

UNIVERSITY OF ALBERTA

DOPAMINE AND ANTIDEPRESSANT DRUG ACTION

by

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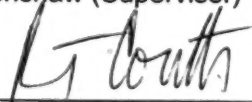
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ABSTRACT

The effects of chronic antidepressant drug treatment on rat behavioural responses to low doses of the dopamine (DA) D₂-like receptor agonists, apomorphine and quinpirole, were assessed in order to investigate how antidepressant drugs mediate their effects *via* the DA system. Chronic s.c. administration of the tricyclic antidepressant (TCA), desipramine (DMI), and the monoamine amine oxidase (MAO) inhibitors, phenelzine (PLZ) and clorgyline (CLG), attenuated apomorphine- and quinpirole-induced locomotor-suppression without changing DA uptake site density. These antidepressants may mediate their effects by decreasing DA autoreceptor function. There was no effect with clomipramine (CMI) and fluoxetine (FLU), potent and selective 5-HT uptake inhibitors, respectively, suggesting that antidepressants which selectively inhibit 5-HT uptake may not mediate their effects by decreasing DA autoreceptor function. Trimipramine (TMP), an antidepressant for which the mechanism of action is currently unknown, also had no effect. PLZ and CLG, but not DMI and CMI, attenuated quinpirole-induced yawning, suggesting that these two behaviours may be mediated by different mechanisms of action and that there may be a difference between the mechanism of action of TCAs and MAO inhibitors. Chronic antidepressant treatment had no effect on locomotor activity and grooming induced by SKF 38393, a D₁-like DA receptor agonist. The attenuation of quinpirole-induced locomotor-suppression following chronic CLG treatment (s.c.) suggested that selective type-A MAO inhibition may be a sufficient condition for mediating the effects of MAO inhibitors. Chronic p.o. administration of moclobemide (MOC) and

brofaromine (BROF) (reversible, selective type-A MAO inhibitors) and of PLZ and CLG (irreversible, nonselective and selective type-A MAO inhibitors, respectively) did not attenuate apomorphine-induced locomotor suppression. The effects of 10 mg kg⁻¹ PLZ were different following s.c. versus p.o. administration despite comparable levels of MAO inhibition, indicating that MAO inhibition is not a sufficient condition for mediating the effects of PLZ on DA autoreceptor function. PLZ at 17 and 30 mg kg⁻¹ p.o. attenuated apomorphine-induced locomotor suppression to the same extent despite a significant difference in the brain levels of PEA and GABA at these two doses. An increase in brain PEA or GABA is not a sufficient condition for mediating PLZ's effects. The effects of a range (0.1 - 30 µM) of concentrations of apomorphine on 15 mM K⁺-stimulated [³H]DA release from nucleus accumbens prisms were assessed. Of these concentrations, only the 3 µM apomorphine reduced the stimulated [³H]DA release.

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ABBREVIATIONS

ADH	aldehyde dehydrogenase
ADR	aldehyde reductase
ANOVA	analysis of variance
L-AAD	aromatic L-amino acid decarboxylase
B _{max}	maximum density of binding sites (in fmol mg ⁻¹ protein)
BROF	brofaromine
CaCl ₂	calcium chloride
CLG	clorgyline
CMI	clomipramine
COMT	catechol-O-methyltransferase
mCPP	<i>m</i> -chlorophenylpiperazine
CON	control
CSF	cerebrospinal fluid
CYP	cytochrome P450
°C	degrees Celsius
d	day(s)
DA	dopamine
DMCMi	desmethyldomipramine
DMI	desmethyldimipramine
DOPAC	3,4-dihydroxyphenylacetic acid
EC	electrochemical detection(or)
ECD	electron capture detection(or)
EDTA	ethylenediaminetetraacetic acid
FLU	fluoxetine
fmol	femtomole(s)
g	gram(s)
GC	gas chromatography
h	hour(s)
HP	Hewlett Packard
HPLC	high-pressure liquid chromatography
HVA	homovanillic acid
6-OHDA	6-hydroxydopamine
5-HIAA	5-hydroxyindole-3-acetic acid
7-OH-DPAT	R(+)-7-hydroxy-2-(di- <i>n</i> -propylamino)tetralin
8-OH-DPAT	(±)-8-hydroxy-2-(di- <i>n</i> -propylamino)tetralin
5-HT	5-hydroxytryptamine; serotonin
i.d.	internal diameter
i.p.	intraperitoneally
I.S.	internal standard
K ⁺	potassium
KCl	potassium chloride
K _d	dissociation constant (in nM)
kg	kilogram(s)

K _i	inhibition constant
MgSO ₄	magnesium sulphate
mg	milligram(s)
ml	milliliter(s)
mm	millimeter(s)
mM	millimolar
min	minute(s)
M	molar
MAO	monoamine oxidase
MOC	moclobemide
ng	nanogram(s)
nM	nanomolar
Ni	nickel
NPD	nitrogen-phosphorus detector
Na ⁺	sodium
NaCl	sodium chloride
NA	noradrenaline
NFLU	norfluoxetine
NTMP	nortrimipramine
%	percent
pg	picogram(s)
p.o.	per os, orally
PfPh-OH	pentafluorophenol
PLZ	phenelzine
PEA	2- or β-phenylethylamine
SAL	saline
s.c.	subcutaneous
TCA	tricyclic antidepressant
TFMPP	trifluoromethylphenylpiperazine
TMP	trimipramine
Tris	Tris buffer
TRP	tryptophan
μg	microgram(s)
μl	microliter(s)
μM	micromolar
VEH	vehicle

CHAPTER 1

General Introduction: DA and The Pharmacotherapy of Depression.

1.1. Introduction

The serendipitous discovery in the 1950s that iproniazid, a drug used to treat tuberculosis, led to an elevation of mood in tuberculosis patients (Selikoff *et al.*, 1952; Crane, 1957) was instrumental in providing the impetus for a better understanding of and further development of antidepressant treatments. Reports by Bloch *et al.* (1954) and Selikoff *et al.* (1952) that this anti-tuberculosis drug had mood elevating effects in these patients led to subsequent studies (Crane, 1957; Kline, 1958) which demonstrated that iproniazid was effective as an antidepressant. It was later reported that the enzyme monoamine oxidase (MAO) was inhibited by iproniazid both *in vitro* and *in vivo* in the brain (Zeller, 1963). Since this drug was known to inhibit MAO, which is the principal enzyme involved in metabolizing the biogenic amines, other MAO inhibitors were developed and used in the treatment of depression.

About this same time, it was observed (Kuhn, 1958) that the tricyclic compound imipramine, which was being tested as a possible antipsychotic, elevated the mood of schizophrenic patients. It was later determined that imipramine inhibited the neuronal uptake of noradrenaline (NA) and serotonin (5-hydroxytryptamine, 5-HT), thus increasing the availability of these biogenic amines in the synaptic cleft. Consequently, it was proposed that this effect was responsible for the antidepressant effect of imipramine (Glowinski and Axelrod, 1964; Carlsson *et al.*, 1968).

The ability of MAO inhibitors and TCAs to increase the availability of the

biogenic amines by preventing their metabolism and inhibiting their neuronal uptake, respectively, led to the development of the biogenic amine theory of depression. This theory postulates that depression is caused by a functional deficit of biogenic amines, primarily NA and 5-HT, and that antidepressant treatments alleviate the symptoms of depression by increasing the availability of these biogenic amines in the synaptic cleft, and therefore, at the postsynaptic receptor. Support for this theory also derives from the fact that drugs such as reserpine and α -methyl-p-tyrosine which, respectively, deplete amines from the neuron (Pletscher *et al.*, 1956) or inhibit their synthesis (Chessin *et al.*, 1956; Cooper *et al.*, 1991), can produce depression.

There are, however, problems with this theory. For example, cocaine, a drug which inhibits the neuronal uptake of DA, is not effective as an antidepressant. In addition, there are novel antidepressant drugs, such as iprindole and mianserin, which do not inhibit the uptake of or prevent the metabolic breakdown of biogenic amines, yet are clinically effective as antidepressants. Another problem with this theory is the discrepancy between the time in which the biochemical effects of these drugs occur and the time with which the observed alleviation/remission of the depressive symptoms occur. Although the biochemical effects of these drugs occur almost immediately after their administration, it can take 2-3 weeks before the clinical effects of antidepressant therapy are evident (Maj *et al.*, 1984). These discrepancies encouraged researchers to search for the common biological changes which emerge following chronic treatment with antidepressant drugs.

1.2. Antidepressant Treatments

1.2.1. Monoamine Oxidase Inhibitors:

Monoamine oxidase (MAO) is a flavin-containing enzyme which is located both extraneuronally and intraneuronally. It is bound to the outer mitochondrial membrane of both neuronal and non-neuronal cell types (Berry *et al.*, 1994). MAO catalyzes the oxidative deamination of biogenic amines to their corresponding aldehydes (Cooper *et al.*, 1991). Along with the aldehyde intermediate, hydrogen peroxide and ammonia are produced as byproducts. The aldehyde intermediate is then rapidly metabolized by aldehyde dehydrogenase to the corresponding acid. In addition to inhibiting the oxidative deamination of monoamines, some MAO inhibitors also inhibit the activity of other enzymes such as diamine oxidase, 5-hydroxytryptophan decarboxylase, and dopamine β -hydroxylase (Cooper *et al.*, 1991).

MAO is known to exist in the form of two isoenzymes, MAO-A and MAO-B. The amino acid sequences of the two different isoenzymes MAO-A and -B exhibit a 70% homology (Bach *et al.*, 1988; Powell *et al.*, 1989) and are encoded by separate genes on the X chromosome (Kochersperger *et al.*, 1986). Overall, it appears that the distribution of the MAO enzyme in human tissue is similar to that found in other species (Berry *et al.*, 1994). Although both types of the MAO enzyme are found in humans and rats, it has been determined that while the human brain contains a higher amount of MAO-B, MAO-A is found in a greater abundance in the brain tissue of rats (Fowler *et al.*, 1980; Garrick and Murphy, 1980; Azzaro *et al.*,

1985; Kalaria *et al.*, 1988). As a result of these relative differences regarding the two types of MAO enzymes in the brains of the two species, DA is primarily a substrate for MAO-B in humans versus MAO-A in rats. Liver tissue, on the other hand, tends to exhibit less difference between the two species with respect to the abundance of MAO-A versus MAO-B (Garrick and Murphy, 1980).

MAO types A and B differ with respect to their sensitivity to inhibitors and to the substrates which they may metabolize. For example, clorgyline (Figure 1) (at low doses) is a selective, irreversible inhibitor of MAO-A while (-)-deprenyl (at low doses) is an irreversible inhibitor which selectively inhibits MAO-B. NA and 5-HT are preferential substrates for MAO-A while MAO-B preferentially metabolizes 2-phenylethylamine (PEA) and benzylamine. Some MAO inhibitors, such as PLZ (Figure 1), can inhibit both enzymes equally, while there are some amines, such as DA, tyramine and tryptamine, which appear to be substrates for either type of MAO.

MAO inhibitors are widely effective in treating depressed patients. They may be particularly effective in treating panic disorder as well as atypical depression which is characterized by increased appetite or weight gain, hypersomnia, mood reactivity and rejection sensitivity (Quitkin, 1979, 1990, 1991; Frances *et al.*, 1995; Hyman *et al.*, 1995). Side-effects associated with the use of MAO inhibitors include restlessness and insomnia; these are more common with tranylcypromine than with PLZ. Anticholinergic-like effects, such as blurred vision, dry mouth, urinary retention and constipation, can also occur but are less severe than when seen following administration of the TCAs. Unlike the situation with the TCAs, these adverse

effects of MAO inhibitors are apparently not due to antagonism at muscarinic receptors (Hyman *et al.*, 1995). Baldessarini (1985) suggests that since these functions represent a balance between cholinergic and sympathetic autonomic functions, the anticholinergic side-effects may be due to a deficiency of vagal, pelvic and other muscarinic parasympathetic functions or from an excess of adrenergic, sympathetic function. Orthostatic hypotension and weight gain are also quite common with the MAO inhibitors.

The most dangerous side-effect associated with the irreversible MAO inhibitors is the potentially fatal pressor effect which can result from an interaction with certain foods. This hypertensive crisis, also known as the "cheese effect" occurs when foods containing tyramine or other sympathomimetic agents are taken concurrently with MAO inhibitor treatment. As Blackwell (1963) first reported, the side-effects arising from concomitant MAO inhibitor treatment and ingestion of foods with tyramine may include hypertension, tachycardia, palpitations, occipital headache, nausea and vomiting. These reactions, which may also include dilated pupils, pyrexia and motor agitation, can develop within 20 minutes to an hour after ingestion of tyramine-containing food (Krishnan, 1995). In some cases, the severity of the side-effects is such that they may lead to cerebral hemorrhage and even death, although this is rare (0.01%-0.02% for tranylcypromine).

Due to the potentially dangerous or even fatal nature of these side-effects, certain dietary restrictions are placed on patients taking MAO inhibitors. These include the restricted intake of cheese, wine, fermented products, brewer's yeast and fava beans, to name a few (Krishnan, 1995). In addition to certain foods, it is

usually recommended that patients taking MAO inhibitors avoid sympathomimetic drugs. As a consequence of these restrictions, patient compliance may be decreased when they are taking irreversible MAO inhibitors (Priest *et al.*, 1995). When MAO activity, particularly MAO-A found in the gut, is inhibited, the metabolic breakdown of tyramine is prevented. Tyramine is taken up by noradrenergic neurons via the NA transporter located in the cytoplasmic membrane and is then taken up into synaptic vesicles, displacing NA into the cytosol. NA is then available to reach and contract blood vessels. The result of this effect is an increase in systolic blood pressure (Cooper, 1989).

PLZ and CLG are both examples of irreversible MAO inhibitors. However, whereas CLG is selective for MAO type-A, PLZ is nonselective, inhibiting both MAO type-A and -B. PLZ is interesting in that it not only inhibits MAO but is also a substrate for it (Clineschmidt and Horita, 1969a,b). CLG is an effective antidepressant (Murphy *et al.*, 1981), however, it has a high potential for inducing the "cheese effect" (Blackwell, 1963). (-)-Deprenyl, on the other hand, which is a selective inhibitor of MAO-B at therapeutic doses (Knoll, 1976, 1978), has not been reported to be a particularly effective antidepressant (Mann *et al.*, 1984) but is reported as being free of the "cheese effect". Due to the potential seriousness of side-effects and the necessary dietary restrictions associated with irreversible MAO-A inhibitor antidepressants, there developed an interest in developing MAO-A inhibitors which are reversible in their action, potentially leading to less serious side effects.

MOC (Figure 1) is a reversible, selective type-A MAO inhibitor. As a result of its reversible nature, there is less concern, compared with the irreversible MAO inhibitors, regarding the potential for this drug to interact with sympathomimetic amine drugs or foods containing tyramine to induce a hypertensive or "cheese effect". Furthermore, recovery of the MAO enzyme is complete within 24 hours; this is beneficial when a new drug must be subsequently administered, resulting in a minimal chance of drug-drug interactions and perhaps dangerous consequences. This is an advantage the reversible MAO inhibitors have over the irreversible MAO inhibitors; in the latter case, a new drug therapy should not be introduced for up to at least two weeks, the time required for synthesis of new MAO enzyme to occur. Recovery of the MAO enzyme activity requires the actual synthesis of new enzyme following treatment with irreversible MAO inhibitors due to the covalent and irreversible bonding of irreversible MAO inhibitors to the active site of the MAO enzyme. Thus, the potential for developing serotonin syndrome, characterized by hypomania, confusion, myoclonus, hyperactivity, tremor, hyperthermia and sedation does not appear to be as great following the coadministration of selective serotonin reuptake inhibitors (SSRIs) (or other serotonergic agents) and reversible MAO inhibitors compared to the situation with irreversible MAO inhibitors (Mayersohn and Guentert, 1995). There is also less chance of adverse effects occurring when replacing the reversible MAO inhibitors, such as MOC, with TCAs or coadministering the TCAs (Amrein *et al.*, 1992; Dingemanse *et al.*, 1995).

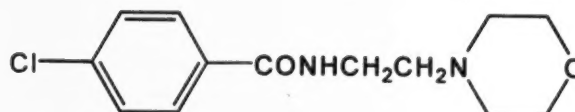
MOC appears to have almost no affinity for dopaminergic, adrenergic, serotonergic, muscarinic, benzodiazepene or opioid receptors (Da Prada *et*

al., 1989). This feature of MOC probably accounts for its low incidence of side-effects and may also reduce the potential for adverse drug-drug interactions. In a report published by Hilton *et al.* (1995), MOC was reported as eliciting no increased risk of hypertensive crisis, orthostatic hypotension, hepatotoxicity or any other side-effects common to the irreversible MAO inhibitors.

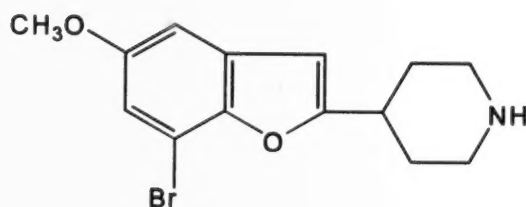
Overall, side effects have been reported in less than 0.2% of a population of approximately 780,000 subjects who had been exposed to MOC between 1986 and 1993. Not only has MOC been shown to have fewer side-effects than the irreversible MAO-inhibitors, TCAs and the SSRIs, it has also been demonstrated to have an efficacy as an antidepressant which is comparable to these other antidepressant treatments (Paykel, 1995; Priest *et al.*, 1995). Dizziness, insomnia and nausea have been shown to occur only slightly more (3%) than with placebo. In addition, MOC appears to be effective in the treatment of social phobias (Versiani *et al.*, 1992) and panic disorder (Johnson *et al.*, 1994). An added advantage of MOC is that, due to the reduced number of side-effects and restrictions placed upon the patient, compliance may be increased.

BROF (Figure 1) is another reversible, selective type-A MAO inhibitor which has demonstrated good efficacy as an antidepressant (Schiwy *et al.*, 1989; Verhoeven, 1995). BROF has also been shown (Garcia-Borreguero *et al.*, 1992; Bakish *et al.*, 1993) to be very effective in treating panic disorder, with therapeutic results comparable to that of CMI. Its efficacy in the treatment of social phobia has also been demonstrated (Lott *et al.*, 1997). Thus, the reversible MAO inhibitors

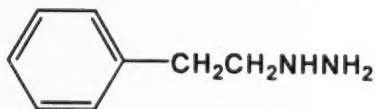
Moclobemide



Brofaromine



Phenelzine



Clorgyline

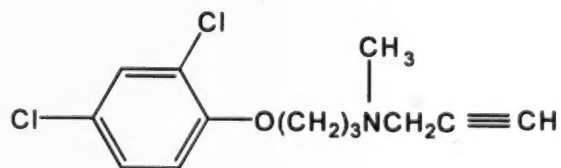


Figure 1. Structures of MAO inhibitors used in this thesis.

appear to be as effective as other antidepressants and have a very good safety profile.

1.2.2. Tricyclic Antidepressants

The TCAs served as the main source of antidepressant therapy from the 1960s to the 1980s in the United States (Potter *et al.*, 1995). Although the tricyclic moiety of imipramine, known as iminodibenzyl, was actually synthesized in 1889 (Baldessarini, 1985), it was not until 1948 that imipramine was synthesized. Due to their observed sedative properties in animals, iminodibenzyl derivatives such as imipramine, which is a structural analog of chlorpromazine (Hyman *et al.*, 1995), were used to treat psychotic patients. Kuhn (1958), in an attempt to treat a psychotic patient with imipramine, noticed a marked improvement in a population of psychotic patients characterized as depressed. He subsequently administered imipramine to a population of patients diagnosed with depression in the absence of psychosis and found a significant improvement in these depressed patients.

As mentioned previously, the biogenic amine theory of depression arose, in part, based upon the observation of the ability of the TCAs to inhibit the reuptake of the biogenic amines (which is the primary method of inactivating the biogenic amines) from the synaptic cleft into their respective nerve terminals. The inhibition constants (K_i 's) listed in Table 1 describe the potency of the TCAs and SSRIs used in this thesis to inhibit the uptake of biogenic amines into rat brain synaptosomes (Richelson and Pfenning, 1984; Bolden-Watson and Richelson, 1993). Tertiary

K _i ± S.E.M. (nM)			
Antidepressant	[³ H]DA	[³ H]5-HT	[³ H]NA
DMI	5200 ± 700 ¹	340 ± 60 ¹	0.9 ± 0.2 ¹
	11000 ± 2000 ²	180 ± 10 ²	0.61 ± 0.07 ²
CMI	1800 ± 200 ¹	5.4 ± 0.2 ¹	28 ± 7 ¹
FLU	1600 ± 200 ¹	1.2 ± 1 ¹	280 ± 70 ¹
	3050 ± 70 ²	14 ± 3 ²	143 ± 6 ²
NFLU	1100 ± 200 ²	25 ± 3 ²	410 ± 30 ²
TMP	3400 ± 400 ¹	2500 ± 200 ¹	510 ± 60 ¹

Table 1. Inhibitory effect of TCAs and SSRIs used in this thesis on the uptake of biogenic amines. Data shown are the inhibitor constants (K_i's) for blockade of [³H]DA, [³H]5-HT and [³H]NA uptake into rat brain synaptosomes. Data are from Richelson and Pfenning (1984)¹ and Bolden-Watson and Richelson (1993)².

amine TCAs (e.g. imipramine and amitriptyline) are more potent at inhibiting 5-HT uptake while their secondary amine metabolites (DMI and nortriptyline, respectively) are more potent NA uptake inhibitors. As a consequence, no TCA can ever be considered to act as a selective inhibitor of 5-HT uptake since the secondary amine metabolites of the tertiary amines inhibit the uptake of NA.

The secondary amine TCAs have a better safety profile and are better tolerated than the tertiary amine TCAs due to their relatively low binding affinity for receptor systems involved in the production of side effects. The tertiary amines have a higher affinity for histaminergic, α -adrenergic and muscarinic receptors compared with the secondary amine TCAs (Potter *et al.*, 1995) and, therefore, have a greater propensity for causing side effects. Adverse side-effects associated with the TCAs include sedation, anticholinergic side effects, weight gain, orthostatic hypotension, cardiotoxicity and photosensitivity. As with the MAO inhibitors, there is also the potential for a fatal overdose with the TCAs.

Sedation is most likely due to the blockade of α -adrenergic and/or histaminergic receptors. Of the TCAs, TMP as well as doxepin and amitriptyline cause the greatest amount of sedation while DMI and protriptyline cause the least (Kaplan and Saddock, 1996). Anticholinergic side-effects are due to the blockade of muscarinic cholinergic receptors and may include dry mouth, urinary retention, constipation, blurred vision and nasal congestion. DMI seems to induce fewer anticholinergic side-effects than do the other TCAs. The blockade of α_1 -adrenergic receptors may also lead to orthostatic hypotension. Cardiovascular side-effects of the TCAs can include tachycardia, hypertension, arrhythmias, and heart block

(Hyman *et al.*, 1995). These potentially fatal side-effects are believed to be the result of an inhibition of fast Na^+ channels and the Na^+/K^+ pump and are the main contributors to the narrow therapeutic index of the TCAs and, consequently, their cardiotoxicity in overdose (Preskorn and Irwin, 1982). In addition, this effect on Na^+ channels is also believed to contribute to the lowered seizure threshold which may occur with TCAs (Preskorn and Fast, 1992).

1.2.3. Selective Serotonin Reuptake Inhibitors

As the name implies, the SSRIs selectively inhibit the uptake of 5-HT without affecting the uptake of DA or NA into their respective nerve terminals. These drugs also have very little affinity for muscarinic, serotonergic, adrenergic and histaminergic receptors (Thomas *et al.*, 1987). As a consequence, they are virtually devoid of the anticholinergic side-effects and the orthostatic hypotension characteristic of the TCAs and irreversible MAO inhibitors. More importantly, the SSRIs do not have the potential for cardiotoxic effects or fatal overdose that the TCAs do. The SSRIs, however, are not completely free of the potential to cause side-effects.

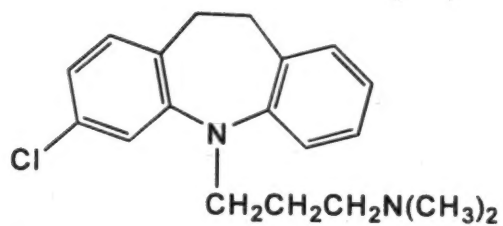
The SSRIs can cause anxiety and nervousness (initial increase), insomnia, sexual dysfunction and, more commonly, gastrointestinal upset such as nausea and loss of appetite and/or weight loss (Hyman *et al.*, 1995). Because some of the SSRIs are also highly protein bound, drug-drug interactions can also occur because other concurrently administered protein-bound drugs may be displaced from carrier

proteins leading to possible toxic levels (Hyman *et al.*, 1995; Preskorn, 1996). One potentially fatal side effect which can result from the interaction of SSRIs and other agents which can increase 5-HT availability, such as MAO inhibitors or tryptophan, is the serotonin syndrome. This results from an excess of 5-HT and may be characterized by diarrhea, confusion, sedation, hypomania, hyperactivity, myoclonus, tachycardia, hypertension, tremors, and fever. It can, however, lead to hyperthermia, convulsions, coma and even death.

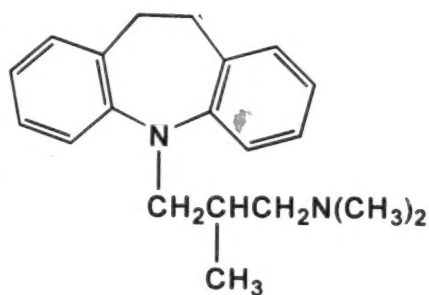
Of the SSRIs, citalopram is the most selective inhibitor of 5-HT, while paroxetine is the most potent (Hyttel, 1982; Thomas *et al.*, 1987; Boyer and Feigner, 1991). FLU (Figure 2) is desmethylated to NFLU (norfluoxetine, N-desmethylfluoxetine). NFLU is a more potent SSRI than FLU and its steady-state plasma concentration has also been found to be 2-3 fold greater than that of its parent compound (Kelly *et al.*, 1989; Preskorn *et al.*, 1991). NFLU also has a much longer half-life (7-15 days) than FLU (2-4 days) (Preskorn, 1993; Hyman *et al.*, 1995). Consequently, NFLU contributes to the clinical effects of FLU.

One advantage of this long half-life is that the potential for withdrawal side effects is not as great if administration of the drug is suddenly terminated rather than slowly titrated. However, this long half-life requires a longer (5 weeks) washout before initiating treatment with any other drug, especially MAO inhibitors, in order to prevent drug-drug interactions (e.g. the serotonin syndrome in the case of the MAO inhibitors) (Steiner and Fontaine, 1986; Sternbach, 1988; Spina and Perucca, 1994; Leonard, 1995).

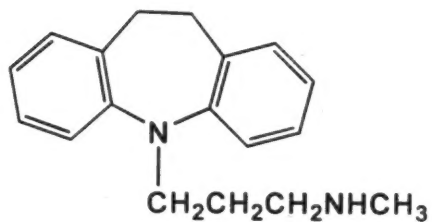
Clomipramine



Trimipramine



Desipramine



Fluoxetine

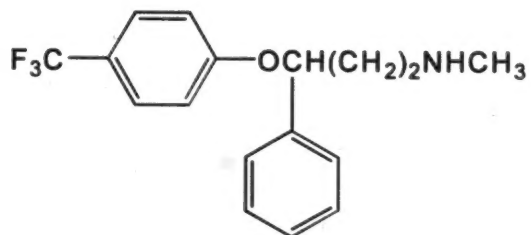


Figure 2. Structures of the TCAs (DMI, CMI and TMP) and the SSRI (FLU) used in this thesis.

Desmethylocitalopram and didesmethylcitalopram are metabolites of citalopram. They are both more potent than the parent drug at inhibiting the uptake of NA (Hyttel, 1993), but because they exist in plasma at concentrations no more than $\frac{1}{2}$ - $\frac{1}{3}$ of the levels of citalopram, it is thought that these metabolites may not contribute much to the overall therapeutic activity of the parent drug (Milne and Goa, 1991). The metabolite of sertraline, N-desmethylsertraline, is less potent as a 5-HT uptake inhibitor (Heym and Koe, 1988; Bolden-Watson and Richelson, 1993; Cusack *et al.*, 1994) than is its parent compound. Preskorn *et al.* (1993) suggest that since this metabolite only reaches plasma levels 1.5 times that of sertraline, it is unlikely that it would mediate any of the clinical effects of sertraline. With respect to inhibition of 5-HT uptake, the metabolites of paroxetine and fluvoxamine are much less potent than the parent drugs and appear to be clinically inactive even though the levels of these metabolites may exceed those of the respective parent compound (Doogan and Caillard, 1988; Tulloch and Johnson, 1992).

SSRIs have been used in combination with TCAs in the case of treatment-resistant depression and also in an attempt to decrease the latency between initiation of antidepressant treatment and observation of therapeutic effects (Eisen *et al.*, 1989; Nelson *et al.*, 1991; Hyman *et al.*, 1995; Rosenbaum *et al.*, 1995). In this context, DMI has been coadministered with FLU (Eisen *et al.*, 1989; Nelson *et al.*, 1991). It has been observed that patients receiving this combination therapy showed a more rapid clinical response than those receiving DMI alone. Since FLU inhibits the activity of the cytochrome P450 (CYP) enzyme (Otton *et al.*, 1993) which

metabolizes DMI, monitoring plasma DMI levels is mandatory in order to prevent any side effects which may arise due to elevation of levels of the TCA (Westermeyer, 1991; Biederman *et al.*, 1992; Hyman *et al.*, 1995). It has been suggested that the dose of the TCA be reduced when coadministered with an SSRI due to the inhibition of TCA metabolism by the SSRI (Westermeyer, 1991; Hyman *et al.*, 1995).

One important property of the SSRIs is their ability to inhibit CYP hepatic isoenzymes (Otton *et al.*, 1993). The SSRIs are both inhibitors of and substrates for the CYP isoenzymes (Tollefson *et al.*, 1995). One consequence of their interaction with these metabolic enzymes is the potential for elevating the concentration of any coadministered drug which is metabolized by the same CYP enzyme.

FLU and paroxetine are both potent inhibitors of CYP2D6 (Crewe *et al.*, 1992; Otton *et al.*, 1993). To a lesser extent, citalopram and sertraline also inhibit CYP2D6. FLU is also believed to inhibit at least one of the CYP2C enzymes as well as CYP3A4 (Lemberger *et al.*, 1988; Shader *et al.*, 1994). Of all the SSRIs, fluvoxamine is the weakest inhibitor of CYP2D6 but a more potent inhibitor of CYP1A2 (Brosen *et al.*, 1993; Perucca *et al.*, 1994). Interestingly, the metabolites of SSRIs appear to be specific for the same CYP enzymes as their respective parent compound (Preskorn, 1993). The M2 metabolite of paroxetine is about one third as potent as the parent drug at inhibiting CYP2D6. NFLU is equal in potency to paroxetine's metabolite but slightly more potent and selective as an SSRI than

its own parent compound, FLU (Crewe *et al.*, 1992). NFLU is more potent than FLU at inhibiting CYP 3A4. Sertraline is thought to inhibit CYP 3A3/4 but not as potently as NFLU. Desmethylsertraline inhibits CYP2D6 in a manner similar to its parent compound, sertraline (Crewe *et al.*, 1991; Brosen *et al.*, 1993; von Moltke *et al.*, 1994).

Due to their greater pharmacological specificity, the SSRIs, overall, tend to have fewer side-effects than the TCAs. However, due to their ability to inhibit the CYP enzymes in the liver, there is the potential for drug-drug interactions when the SSRIs are used in conjunction with other drugs. Furthermore, even after discontinuation of a drug with a long half-life, such as FLU, the CYP enzymes it inhibits will still be affected and thus the clearance of the other drugs metabolized by the respective CYP enzymes will be delayed (Lane *et al.*, 1995). Therefore, it is important to be aware of the specific CYP enzymes inhibited by the SSRI and which ones metabolize any other drug which is used concurrently with the SSRI in order to prevent any toxic effects.

1.3. DA and Depression

That DA may be involved in depression was first suggested by Randrup *et al.* (1975). DA is believed to play an important role in mediating the rewarding effects of stimuli. Anhedonia, the inability to experience pleasure from stimuli which are normally rewarding, is a primary feature of depression. Thus, antidepressants may alleviate depression by increasing DA neurotransmission.

Several lines of evidence suggest the importance of DA in mediating the rewarding effects of stimuli and indicate that this amine may be important in the etiology and treatment of depression. For instance, experiments suggest that the meso-accumbens (mesolimbic) DA pathway is important in mediating the rewarding properties of stimuli. Several studies have demonstrated that intracranial self-stimulation (ICSS) elicited by electrodes implanted in the ventral tegmental area (VTA) is mediated by the mesocorticolimbic DA pathway (Le Moal and Simon, 1991). ICSS results in an increase in the synthesis, release and metabolism of DA (Simon *et al.*, 1979; Fibiger *et al.*, 1987; Philips *et al.*, 1987) in the nucleus accumbens and striatum. Furthermore, 6-OHDA-induced lesions of these DA pathways decrease ICSS in the VTA. It has also been demonstrated that rats will self-administer drugs such as cocaine and amphetamine, which are known to mediate their effects via DA release, and that 6-OHDA-induced lesions in the nucleus accumbens will ultimately decrease the responding by animals receiving these drugs (Le Moal and Simon, 1991).

The fact that agents such as haloperidol, a D₂ DA receptor antagonist, or

reserpine, which depletes DA from the neuron, can decrease DA neurotransmission and induce depressive features in humans (Belmaker and Wald, 1979) also suggests that DA plays an important role in both the etiology and treatment of depression. Treatment with low doses of D₂ DA receptor antagonists, such as levosulpiride or amisulpiride, which are believed to be selective for presynaptic DA autoreceptors has also been reported to be an effective method of antidepressant therapy (del Zompo *et al.*, 1990; Lecrubier *et al.*, 1997). Blockade of the D₂ DA presynaptic autoreceptors results in an increase in DA neurotransmission whereas antagonism of postsynaptic DA receptors results in a decrease.

A reduction in the CSF concentration of the DA metabolite, HVA, has been found in suicidal patients (Oreland *et al.*, 1981; Traskman *et al.*, 1987). In addition, a negative correlation has been reported between CSF levels of HVA and scores obtained on the Hamilton Depression Rating Scale (Peabody *et al.*, 1987). The finding that HVA levels are decreased in the CSF of depressed patients compared to controls serves as important evidence for the possible role of DA in depression (Mendels *et al.*, 1972; Roy *et al.*, 1985, 1989; Reddy *et al.*, 1992). Serum levels of DA and DA β -hydroxylase have been reported to be higher and lower, respectively, in patients diagnosed as having depression with psychotic features such as hallucinations or delusions (Rothschild *et al.*, 1987; Sapru *et al.*, 1989). Both plasma and CSF HVA levels have also been reported to be significantly higher in psychotic depressives than nonpsychotic depressives (Aberg-Wistedt *et al.*, 1985; Mazure *et al.*, 1987).

Additional evidence which seems to support the role of DA in mediating the effects of antidepressant drugs is the ability of antidepressant drugs with different biochemical effects to modulate the functional responses to DA receptor agonists (Willner, 1997). Antidepressant drugs which have different mechanisms of action, such as DMI and FLU, as well as buspirone, a 5-HT_{1A} receptor agonist, have been reported to increase extracellular DA levels in the prefrontal cortex of rats (Tanda *et al.*, 1994; Gobert *et al.*, 1997). These findings lend support to the possible role of DA in mediating the effects of antidepressant drugs.

Chronic treatment with pramipexole, a new D₂/D₃ DA receptor agonist, has been reported to reverse the effects of chronic mild stress (Mierau and Schingnitz, 1992), reduce the immobility time in the forced-swimming test and increase D-amphetamine- or quinpirole-induced locomotor activity (Maj *et al.*, 1997). Furthermore, the DA receptor agonist and DA uptake inhibitor, bromocriptine (Silverstone, 1984; Sitland-Marken *et al.*, 1990) and bupropion (Fernberg, 1990), respectively, have been shown to be effective antidepressants. Bromocriptine has also been reported to be very effective when coadministered with other antidepressants to treatment-resistant patients (Inoue *et al.*, 1996). In addition, Nielsen (1986) has reported that the apomorphine-induced inhibition of DOPA accumulation in rat striatum is attenuated following chronic administration of bupropion. An enhancement of DA neurotransmission has been thought to underlie the improvement reported in depressed patients following augmentation therapy with psychostimulants (Hyman *et al.*, 1995). Symptoms such as

anhedonia, anxiety and depressed mood which are associated with depression are also associated with stimulant abuse and withdrawal (Mann and Kapur, 1995). Since the effects of stimulants such as amphetamine are thought to be mediated through the mesolimbic DA system, it may be that this DA pathway may play a role in the etiology and treatment of depression.

In an attempt to elucidate the possible role of DA in mediating the clinical effects of antidepressants, studies have been conducted in which the effects of chronic antidepressant treatment on the behavioural responses to DA agonists have been observed in rats. A potentiation of the behavioural responses to high doses of DA receptor agonists, such as apomorphine and amphetamine, have been observed following chronic treatment with both TCAs and MAO inhibitors (Spyraki and Fibiger, 1981; Martin-Iverson *et al.*, 1983; Serra *et al.*, 1990). Both neuropharmacological and electrophysiological data indicate that DA autoreceptor-mediated responses may be decreased following chronic antidepressant treatment (Chiodo and Antelman, 1980a,b; Antelman and Chiodo, 1981; Nielsen, 1986). Chronic antidepressant treatment in rats has been reported to potentiate the amphetamine-induced increase in extracellular DA in the nucleus accumbens and attenuate the apomorphine-induced decrease in extracellular DA in the nucleus accumbens and striatum (Nielsen, 1986; Nomikos *et al.*, 1991).

An attenuation of the behavioural responses of low doses of DA receptor agonists, believed to be selective for DA presynaptic autoreceptors, has also been demonstrated following chronic antidepressant treatment (Serra *et al.*, 1979; Maj *et*

al., 1989). Data from two of the studies presented in this thesis report an attenuation of the locomotor-suppressant response to low-dose DA receptor agonists following chronic antidepressant treatment (Allison *et al.*, 1993, 1995).

Animal models of depression have also served as support for the role of DA in depression. In the chronic mild stress animal model of depression, rats are chronically exposed to an unpredictable mild stressor such as a change in their cage mate. Exposure to this stressful stimulus results in a reduced responsiveness to rewarding stimuli (anhedonia), such as a sucrose solution, which is reversed by chronic antidepressant administration (Willner *et al.*, 1992). This effect of the chronic antidepressant treatment is reversed by D₂-like DA receptor antagonists. These results strongly suggest that the DA system is mediating at least some of the effects of antidepressant drugs.

5-HT is another neurotransmitter which has been implicated in both the etiology and treatment of depression. Electrophysiological studies by Blier and de Montigny (1994) have demonstrated that different classes of antidepressants are capable of increasing 5-HT neurotransmission. This has been demonstrated following both acute and chronic administration of antidepressants with different mechanisms of action (Kreiss and Lucki, 1995). Furthermore, a depletion of dietary tryptophan (TRP), the precursor to 5-HT, has been reported to cause a relapse of depression in patients following successful antidepressant treatment (Delgado *et al.*, 1994).

In contrast to findings with the DA metabolite HVA, however, most studies

have reported no difference in the CSF levels of the 5-HT metabolite 5-HIAA in depressed patients compared with controls (Meltzer and Lowy, 1987; Reddy *et al.*, 1992). Low CSF 5-HIAA levels appear to be more related to violent suicide than to depression per se (Faustman *et al.*, 1991). In fact, it has been reported that low CSF 5-HIAA levels are associated with aggression, poor impulse control and impulsive violent behaviour rather than depression (Linnoila and Virkunen, 1992; Virkunen *et al.*, 1994).

The SSRIs are quite often the first choice and most commonly prescribed treatment for depression. This, however, is not due to their greater efficacy as an antidepressant treatment, but is instead due to their safer side-effect profile (Hyman *et al.*, 1995). A reduced latency (approximately one week) of the antidepressant response in depressed patients and an improvement (within one week) in treatment-resistant depressed patients have been reported following the coadministration of an SSRI or MOC and pindolol, a 5-HT_{1A}/β-adrenoceptor antagonist (Artigas *et al.*, 1994, 1996; Blier and Bergeron, 1995). These combination treatment strategies have been reported to exert their therapeutic effects with a rapid onset and with greater efficacy than other antidepressants. This was in contrast to the complete lack of antidepressant effect reported with the combination of pindolol and DMI or TMP, two antidepressants which do not inhibit 5-HT uptake (Blier *et al.*, 1997). Therefore, these results seem to suggest that pindolol potentiates the antidepressant effect of drugs which preferentially alter the function of 5-HT neurons.

It has been suggested that the increased efficacy observed following the coadministration of pindolol and SSRIs may be due to the ability of pindolol to block 5-HT_{1A} presynaptic autoreceptors and therefore prevent the reduction in 5-HT neuronal firing which occurs during the initiation of SSRI treatment. This enhancement of 5-HT neurotransmission appears to result in a rapid and more successful treatment of depression. Since the latency with which remission of depressive features occurs parallels the time required for the recovery of 5-HT neuronal firing following the initiation of SSRI administration, it has been suggested that this time required for the recovery of 5-HT neuronal activity could be the cause of the delayed onset of effect of SSRIs (Blier and Bergeron, 1997). In a recent study, Blier *et al.* (1997) reported a 50% reduction in depressive symptoms within one week of initiation of the combined treatment of pindolol and buspirone, a selective 5-HT_{1A} receptor agonist. Blockade of the 5-HT_{1A} presynaptic autoreceptors by pindolol and the selective activation of postsynaptic 5-HT_{1A} receptors by buspirone may play a part in mediating a more rapid antidepressant response following this combination treatment.

The fact that the efficacy of the SSRIs is comparable to other antidepressants such as the TCAs and MAO inhibitors suggests that not only 5-HT but other neurotransmitters may play a role in mediating the effects of antidepressant treatments (Hyman *et al.*, 1995). Even if all classes of antidepressants mediated their effects via an enhanced activity of one particular neurotransmitter, it is possible that this enhancement could be achieved by the different antidepressants via different mechanisms of action (Maes and Meltzer,

1995).

It has been suggested that the effects of serotonergic drugs may be mediated in part by 5-HT modulation of DA activity. The presence of 5-HT receptors in the VTA where there are cell bodies of DA-containing neurons and in the nucleus accumbens where there are terminals of DA-containing neurons (Pazos *et al.*, 1991; Jacobs and Azmitia, 1992) suggests a possible interaction between DA and 5-HT in mediating the effects of antidepressants. Using positron emission tomography, Tiihonen *et al.* (1996) have reported a decrease in [^{11}C]-raclopride binding to striatal D_2 DA receptors in human striatum following a 14-day treatment with the SSRI citalopram. These investigators suggest that this data may reflect an increase in the levels of striatal DA which may be competing for the D_2 DA receptor. Therefore, the effects of SSRIs may not be as selective for the 5-HT system as initially believed and may mediate their effects in part by modulating DA neurotransmission. Furthermore, buspirone, which is sometimes used to potentiate the therapeutic effects of SSRIs, has been reported to increase extracellular DA but not 5-HT levels in the prefrontal cortex of rats (Tanda *et al.*, 1994; Gobert *et al.*, 1997). This finding suggests that an increase in DA neurotransmission may contribute to the antidepressant effects of buspirone and the SSRIs.

The ability of a variety of antidepressants, each with a different biochemical effect, to modulate the functional responses to DA receptor agonists does not necessarily indicate that the DA system is the final common pathway through which chronically administered antidepressants mediate their effects. Furthermore, the

ability of an antidepressant drug, such as an SSRI,¹ to selectively to alter the neurotransmission of one neurotransmitter as its primary mechanism of action, does not rule out the possibility of another neurotransmitter playing a part in mediating the effects of that antidepressant.

There are several lines of evidence which suggest that the effects of antidepressant drugs may not be mediated by one final common pathway. Reserpine, which has been shown to cause depression in humans, depletes DA, NA and 5-HT. Nonselective MAO inhibitors, such as PLZ, are effective antidepressants capable of preventing the metabolism of DA, NA and 5-HT. The fact that there are several subtypes of depression which appear to respond preferentially to different antidepressant treatments suggests that depression is not the outcome of a disruption of any one neurotransmitter system but rather may represent a malfunction in several different neurotransmitter pathways (Brown and Gershon, 1993). A disruption in any one neurotransmitter system may be manifested by specific symptoms predominant over others and may therefore be more treatable by some antidepressants over others. In view of the different subtypes of depression, and the reported efficacy of different types of antidepressants (which appear to be selective for different pathways), it seems unlikely that a single neurotransmitter system could play a role in the etiology and treatment of depression. Thus, a single model approach to explaining the etiology of and treatment of depression seems very unlikely.

1.3.1. DA Synthesis, Release, Uptake and Metabolism

DA is synthesized from the amino acid precursor L-tyrosine by the action of tyrosine hydroxylase (Figure 3) (Wilcox and Gonzales, 1995). L-Tyrosine is produced from dietary L-phenylalanine by phenylalanine hydroxylase, an enzyme found in the liver. L-Tyrosine is taken up into dopaminergic nerve terminals and converted to 3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase. L-DOPA is then taken up into synaptic vesicles where it is subsequently converted to DA by aromatic L-amino acid decarboxylase (also known as DOPA decarboxylase). Aromatic L-amino acid decarboxylase requires pyridoxal phosphate (Vitamin B₆) as a cofactor. Both phenylalanine hydroxylase and tyrosine hydroxylase require molecular O₂, Fe²⁺ and a tetrahydropteridine cofactor (Nagatsu *et al.*, 1964). Tyrosine hydroxylase is the rate-limiting step in the catecholamine biosynthetic pathway. Consequently, manipulation of the activity of this enzyme will ultimately affect the rate of DA synthesis (Cooper *et al.*, 1991). After DA is synthesized, it is packaged into storage vesicles until it is released into the synaptic cleft. In noradrenergic neurons, DA is catalytically converted to NA by the enzyme dopamine β-hydroxylase, which requires molecular O₂ and ascorbic acid as cofactors (Cooper *et al.*, 1991).

DA is released from the presynaptic terminal when an action potential travelling down the axon depolarizes the terminal (Roth and Elsworth, 1995). This causes an increased permeability of the membrane for Ca²⁺. Subsequently, there is an influx of Ca²⁺ ions into and the release of DA from the presynaptic terminal

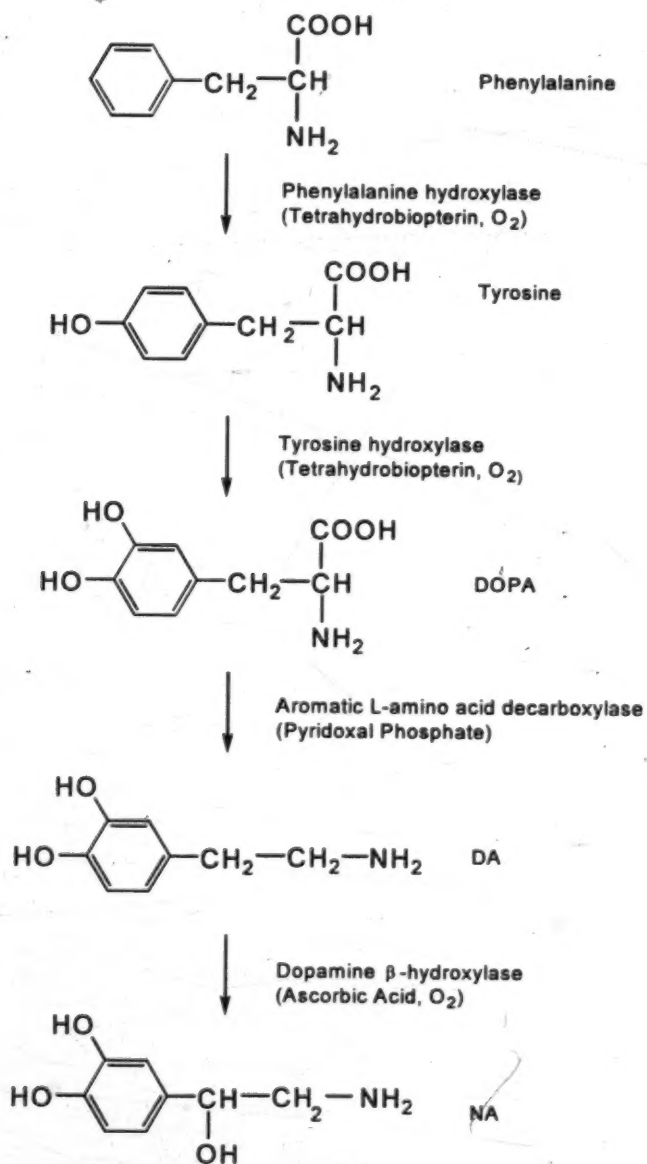


Figure 3. Synthetic pathway of DA and NA. Cofactors are shown in parentheses.

(Baker and Dyck, 1985). The DA released into the synaptic cleft can then act at postsynaptic receptors and subsequently be taken back up into the presynaptic nerve terminal by a DA transporter. The stimulation of somatodendritic DA autoreceptors decreases DA neuronal firing, whereas stimulation of autoreceptors located on the nerve terminal inhibits the synthesis and release of DA (Cooper *et al.*, 1991). Low doses of DA receptor agonists are believed to be selective for presynaptic autoreceptors since their administration results in a decrease in DA synthesis and release (Carlsson, 1975). Low doses of D₂ DA receptor antagonists, on the other hand, tend to selectively block autoreceptors and increase DA release.

The primary mechanism for terminating DA activity is the result of its uptake from the synaptic cleft back into the presynaptic nerve terminal. This is accomplished by a Na⁺-dependent membrane carrier which can transport DA in either direction depending on the concentration gradient (Roth and Elsworth, 1995).

The enzymes important for the catabolism of DA are MAO and COMT (Figure 4). Following reuptake from the synaptic cleft into the neuronal terminal (or even before DA has been stored in vesicles or after it has been released), MAO can convert DA to its acid metabolite, DOPAC. DA can be further converted to HVA by the enzyme COMT. While DOPAC is the major metabolite in rat brain, HVA is the major metabolite in humans (Cooper *et al.*, 1991). DA can also be converted to 3-methoxytyramine (3-MT) directly by COMT. 3-MT can subsequently be converted to 3-methoxy-4-hydroxyphenylacetaldehyde and then ultimately to HVA by the enzymes MAO and aldehyde dehydrogenase (ADH), respectively. In an alternate

pathway, 3-methoxy-4-hydroxyphenylacetaldehyde can be converted to 3-methoxy-4-hydroxyphenylethanol by the action of aldehyde reductase (ADR).

1.3.2. DA Receptors

With the advent of molecular biological techniques, the DA receptors originally identified as either D₁ or D₂ were further classified into five DA receptor subtypes which are categorized as belonging to either the D₁-like or D₂-like families (Seeman, 1995). These two different families can be differentiated based on their affinity for different ligands, their amino acid sequences, signal transduction pathways and anatomical distribution (Schwartz *et al.*, 1992). Both the D₁-like or D₂-like families belong to a superfamily of G-protein-coupled receptors containing seven transmembrane domains. The D₁-like family consists of D₁ and D₅ DA receptors, whereas the D₂-like family consists of D₂, D₃, and D₄ DA receptors. The D₁-like DA receptors are positively coupled to the second messenger adenylate cyclase while the D₂-like receptors are negatively coupled to adenylate cyclase (Civelli *et al.*, 1991).

All G-protein-coupled receptors consist of an amino acid sequence forming seven transmembrane domains of 20-25 hydrophobic amino acids arranged in a helical structure. Each of these hydrophobic spans of amino acids is joined by hydrophilic residues situated in the extracellular and cytoplasmic loops (Schwartz *et al.*, 1992). The alpha (α) helices are arranged so that the N-terminal is situated in the extracellular fluid and the C-terminal is located in the cytoplasm of the neuron.

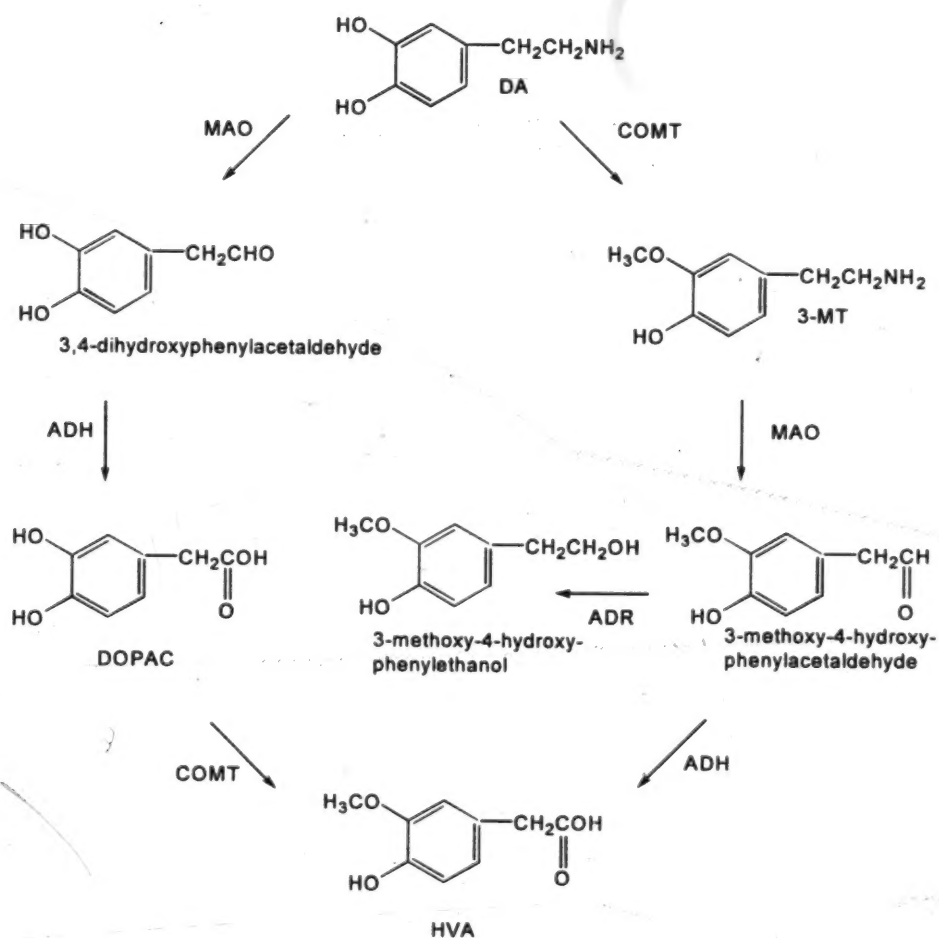


Figure 4. Catabolic pathways of DA.

The third cytoplasmic loop is thought to play an important role in the interaction of the receptor with G-proteins (Dixon *et al.*, 1987; Strader *et al.*, 1989). It is believed that a chain of amino acids situated within the third cytoplasmic loop between the fifth and sixth transmembrane domains and perhaps also some amino acids in the C-terminal region may be responsible for binding of the receptor to the G-protein (Probst *et al.*, 1992). The transmembrane helices are thought to be important for ligand binding. There are three amino acid residues in particular which are thought to interact with catecholamines. In the third transmembrane domain, it is thought that an aspartate (Asp110) forms an ion pair with the protonated amine group of DA. In the fifth transmembrane domain, two serines (Ser 193 and Ser 196) are believed to hydrogen-bond with the two phenol groups of DA (Schwartz *et al.*, 1992).

1.3.3. D₁-like DA Receptors:

The D₁ and D₅ (also referred to as D_{1A} and D_{1B}, respectively) subtypes of the DA receptor family, in contrast to the D₂-like DA receptors, lack introns in the coding regions of their genes. As a result, there are no variants of these two receptor protein subtypes (Weinshank *et al.*, 1991). Currently, there are also no known D₁-like DA autoreceptors. The D₁ DA receptor shows an overall homology to the D₂ DA receptor of 29%, but 44% homology if only the seven transmembrane regions are considered (O'Dowd, 1993). The overall homology between the D₁ and D₅ DA receptors is 50%, with a homology of the transmembrane regions of 80%. DA is 15

times more potent at the D₁ than at the D₂ DA receptor and 10 times more potent at the D₅ versus the D₁ DA receptor (O'Dowd, 1993).

An important difference between the D₁-like DA receptors and the D₂-like DA receptors is the structure of the receptors. The third cytoplasmic loop is shorter and the cytoplasmic carboxy tail is longer in the D₁-like versus the D₂-like DA receptors (Schwartz *et al.*, 1992). The third cytoplasmic loop is believed to be important in mediating the interaction between the receptor and G-proteins (Dixon *et al.*, 1987; Strader *et al.*, 1989). D₁-like DA receptors are coupled to G_s-proteins and stimulate adenylate cyclase whereas D₂-like DA receptors are coupled to G_i-or G_o-proteins and inhibit adenylate cyclase activity (Civelli *et al.*, 1991).

D₁ DA receptor mRNA has been found in the caudate putamen and nucleus accumbens of both human and rat brain tissue (Mengod *et al.*, 1992). It is also found in the olfactory tubercles, Islands of Calleja, amygdala and cortical areas. In neither human nor rat brain tissue has D₁ DA receptor mRNA been localized in the substantia nigra or VTA (Mengod *et al.*, 1992; Civelli, 1995). D₁ DA receptor mRNA also appears to be absent from the globus pallidus in humans and the entopeduncular nucleus in rats. This absence is interesting in view of the presence of D₁ DA receptor binding sites in the globus pallidus and substantia nigra (Mengod *et al.*, 1992). Otherwise, there is a positive correlation between the D₁ DA receptor binding sites labelled with the D₁ DA receptor agonist SCH 23390 and the D₁ DA receptor mRNA distribution in these areas.

The mRNA of the D₅ DA receptor, which has a 10-fold higher affinity for DA

than the D₁ DA receptor, is relatively restricted in its anatomical distribution unlike the D₁ DA receptor which is the most widespread DA receptor in the central nervous system. D₅ DA receptor mRNA is found in the hippocampus, lateral mamillary nuclei, hippocampus and parafascicular nucleus of the thalamus (Tiberi *et al.*, 1991; Meadour-Woodruff *et al.*, 1992). The D₅ DA receptor binds the D₁ selective DA receptor antagonist SCH 23390 and the D₁ selective DA agonist SKF 38393 with an affinity similar to that of the D₁ DA receptor (O'Dowd, 1993). SKF 38393 and SCH 23390 are 100-1000 times less potent at D₂-like DA receptors than at D₁ or D₅ DA receptors (Schwartz *et al.*, 1992).

1.3.4. D₂-like DA Receptors:

The D₂ DA receptor was the first of the DA receptors to be cloned (Bunzow *et al.*, 1988). High levels of D₂ mRNA are found in the caudate putamen, nucleus accumbens, olfactory tubercle, substantia nigra, and VTA. In contrast to the intronless D₁-like DA receptor genes, the D₂-like DA receptor genes contain introns which can be removed during transcription, resulting in different mRNA isoforms or variants of the D₂-like DA receptor subtypes (Giros *et al.*, 1989). As a result, two isoforms of the D₂ DA receptor, D_{2long} (D_{2A}) and D_{2short} (D_{2B}), are produced. These two forms of the D₂ DA receptor, which differ by 29 amino acid residues in the third cytoplasmic loop of the receptor, are the result of differences in the gene structure of these two forms due to the production of multiple mRNA variants (Chio *et al.*, 1990). The D_{2long} DA receptor has an extra 29 amino acid residue in the third

cytoplasmic loop of the receptor compared with that of the short form (Chio *et al.*, 1990).

The amino acid sequences of the transmembrane regions are believed to be important for conferring binding properties to G-protein-coupled receptors. Since the $D_{2\text{long}}$ and the $D_{2\text{short}}$ receptors are homologous with respect to the transmembrane region but differ only by 29 amino acid sequences in the third cytoplasmic loop, it may be that these D_2 DA receptor isoforms interact with different G-proteins resulting in the initiation of different intracellular signals (Dal Toso *et al.*, 1989; Giros *et al.*, 1989). Although both isoforms are found in approximately equal abundance in areas known to express D_2 DA receptor mRNA, such as the basal ganglia, hippocampus, kidney and cerebral cortex, there appears to be a greater abundance of $D_{2\text{long}}$ DA mRNA than of the $D_{2\text{short}}$ form in the striatum, pituitary and olfactory bulb (Neve *et al.*, 1991).

The $D_{2\text{long}}$ DA receptor produces approximately twice the inhibition of cAMP production than that which is observed with the short form of this receptor (Hayes *et al.*, 1992). Dal Toso *et al.* (1989) have demonstrated that both forms appear to bind [^3H]-spiperone, a D_2 DA receptor antagonist, with equal affinity, but, they have also demonstrated that the shorter form inhibits adenylate cyclase to a greater extent than does the $D_{2\text{long}}$ DA receptor. Malmberg *et al.* (1993) have shown that there is a difference in the way in which these two D_2 isoforms (as well as D_2 vs. D_3) bind certain DA receptor antagonists. (+)-AJ76 and (+)-UH232, two putative DA autoreceptor-selective antagonists, have been shown to have equal affinity for

$D_{2\text{short}}$ and $D_{2\text{long}}$ but higher affinity for D_3 than for the D_2 DA receptor. Haloperidol, risperidone and remoxipride had a 4-18 fold higher affinity for D_2 than D_3 DA receptors, with haloperidol and risperidone showing no differentiation with respect to their affinity to the two D_2 isoforms. However, remoxipride, an atypical antipsychotic with few side effects, in addition to raclopride, sulpiride, chlorpromazine, clozapine and thioridazine exhibited an affinity which was 2-3 times greater for $D_{2\text{short}}$ than for the $D_{2\text{long}}$ DA receptor (Malmberg *et al.*, 1993). These compounds, with the exception of remoxipride, showed comparable affinities for D_2 and D_3 DA receptors.

Clozapine has been shown to have a similar binding affinity to $D_{2\text{short}}$ and D_4 DA receptors. Since remoxipride and clozapine, both atypical antipsychotics, have a low potential for causing extrapyramidal side-effects, it has been suggested that their affinity for $D_{2\text{short}}$ versus $D_{2\text{long}}$ DA receptors may be important for this feature of the atypical antipsychotics (Malmberg *et al.*, 1993).

The number of D_3 DA receptors in the brain is lower than the number of D_1 or D_2 DA receptors by approximately two orders of magnitude (Schwartz *et al.*, 1993). DA is approximately 15-20 times more potent at the D_3 DA receptor than at the D_2 DA receptor. Several variants of the rat D_3 DA receptor have been identified, although they are not all established as functional receptors (Giros *et al.*, 1991; Snyder *et al.*, 1991; Schmauss *et al.*, 1992). The rat D_3 DA receptor, which shows an overall homology of 52% with the rat $D_{2\text{long}}$ DA receptor (75% if one considers only the transmembrane regions) (Sokoloff *et al.*, 1990), is found to be localized

mainly in limbic regions in the brain.

It has been suggested that the almost exclusive localization of D_3 DA receptors in limbic areas may indicate a possible role in mediating the clinical effect of antipsychotics (Sokoloff *et al.*, 1990). The D_3 DA receptor is found in the olfactory tubercles and mammillary nuclei of the hypothalamus and its highest density is in the Islands of Calleja and the nucleus accumbens (Landwehrmeyer *et al.*, 1993). These areas receive dopaminergic projections from the VTA, thus suggesting the existence of D_3 DA presynaptic autoreceptors. Although D_3 and D_2 DA receptors coexist in many brain regions, the D_3 DA receptor predominates in the Islands of Calleja whereas D_2 DA receptors predominate in the striatum. There are no D_3 DA receptors in the anterior pituitary gland despite a high level of D_2 expression in this region (Sokoloff *et al.*, 1990).

The human D_3 DA receptor shows an overall homology of 46% with the human D_2 DA receptor (78% if only the transmembrane region is considered) (Schwartz *et al.*, 1992). With respect to the third cytoplasmic loop, there is only about 26% homology between the two receptors (Bouthenet *et al.*, 1991). The homology between the human and rat D_3 DA receptors is 97% in the transmembrane region. There are 46 amino acids which are absent from the third cytoplasmic loop of the human D_3 DA receptor, resulting in a receptor which resembles the $D_{2\text{short}}$ form (Dal Toso *et al.*, 1989). The rat D_3 DA receptor, on the other hand, resembles the $D_{2\text{long}}$ form of the DA receptor. One difference between human and rat D_3 DA receptors is that those of the human but not rat appear to be

coupled to a G-protein. (Sokoloff *et al.*, 1990, 1992a). In a recent study, Griffon *et al.* (1997) have demonstrated that D₂, D₃ and D₄ DA receptors all inhibit cAMP formation and thus inhibit adenylate cyclase consistent with their homologous structures. Apomorphine appears to have a similar affinity for D₂ and D₃ DA receptors (but 5 times higher at D₄) (Van Tol *et al.*, 1991; Sokoloff *et al.*, 1992a). However, quinpirole has an affinity for the D₃ DA receptor which is 50 times more potent than at the D₂ DA receptor. 7-OH-DPAT and PD 128,907 have a 50-100 and 210-fold preference, respectively, for D₃ versus D₂ DA receptors (Sautel *et al.*, 1995).

The identification of 7-OH-DPAT as a selective D₃ DA receptor ligand (Levesque *et al.*, 1992) was important in characterizing this receptor as being located both postsynaptically and presynaptically (as an autoreceptor). Acute administration of the newer, more selective D₃ DA receptor agonist, PD 128,907 (Sautel *et al.*, 1995) has been reported to decrease extracellular levels of DA in the frontal cortex, an area which is innervated by the VTA (Gobert *et al.*, 1996). The selective D₃ DA receptor antagonist (+)-S14297 [(+)-7-(N,N-dipropylamino)-5,6,7,8-tetrahydronaphtho(2,3b)dihydro,2,3-furane)] abolished this effect completely. In addition, PD 128,907 inhibited the firing rate of DA neurons in the VTA following infusion via the femoral vein. This effect was antagonized, in a dose-dependent manner, by (+)-S14297. The selective D₃ DA receptor antagonist (+)-S14297 has also been shown to reduce the 7-OH-DPAT-induced decrease in DA release in the nucleus accumbens although (+)-S14297 itself had no effect on DA levels (Rivet *et*

al., 1994).

As with apomorphine and quinpirole, 7-OH-DPAT shows a biphasic effect on locomotor activity (locomotor suppression at low doses versus locomotor stimulation at high doses), which seems to suggest the differential stimulation of presynaptic autoreceptors versus postsynaptic receptors, respectively (Timmerman *et al.*, 1991; Damsma *et al.*, 1993; Ahlenius and Salmi, 1994; Svensson *et al.*, 1994b). However, it has also been shown (Svensson *et al.*, 1994b) that 7-OH-DPAT decreases locomotor activity at a dose 50 times lower than that required to alter the release or synthesis of DA. Conversely, with apomorphine and quinpirole there is a good correlation between the dose of the drug that decreases locomotor activity and that which decreases DA release (Svensson *et al.*, 1994a). This observation has raised the possibility that the D₃ receptor is located postsynaptically and is inhibitory. Despite this controversy, the possibility of the existence of D₃ receptor mRNA in the substantia nigra and VTA suggests the presence of presynaptic D₃ autoreceptors since the 6-OHDA lesioning of nigrostriatal terminals results in decreased D₃ receptor binding (Sokoloff *et al.*, 1990; Bouthenet *et al.*, 1991; Landwehrmeyer *et al.*, 1993).

The relatively restricted localization of D₃ receptors in the limbic areas of the brain, areas involved in controlling emotion, motivation and reward as opposed to striatal control of motor behaviour and therefore mediation of extrapyramidal side-effects of antipsychotics, has also raised interest in the D₃ DA receptor as a potential target for antipsychotic drugs which might have reduced potential for

eliciting extrapyramidal side-effects.

Atypical antipsychotics, which have a lower propensity to induce extrapyramidal side-effects characteristic of the typical antipsychotics, tend to have a higher affinity for D₃ and D₄ DA receptors compared to the D₂ DA receptor (Sokoloff *et al.*, 1992b; Leysen *et al.*, 1993). The D₄ DA receptor has been shown to have a 10-fold higher affinity for clozapine than do the D₂ or D₃ DA receptors (Van Tol *et al.*, 1991). D₄ DA receptors are found in the amygdala, striatum, frontal cortex, midbrain, medulla and heart (Van Tol *et al.*, 1991, 1992). There appears to be a greater abundance, however, of D₄ DA receptor mRNA in the human central nervous system than that of the rat (Todd and O'Malley, 1993).

With respect to the transmembrane region, the D₄ DA receptor displays 41%, 52% and 51% homology with D₁, D₂ and D₃ DA receptors, respectively (Civelli *et al.*, 1991). The rat gene for the D₄ DA receptor shows a 73% homology with the human gene, but 90% if the transmembrane regions alone are considered (O'Malley *et al.*, 1992).

A number of polymorphic variants of the human D₄ DA receptor have been identified (Van Tol *et al.*, 1992). Each variant contains a different number of repeats consisting of 16 amino acid units located in the third cytoplasmic loop of the receptor. Most (50-70%) humans have 4 repeats (thus resulting in the term D_{4,4} receptor), but this depends on their ethnic background (Petronis *et al.*, 1992; Seeman, 1995). Approximately 14% and 10% have 7 and 2 repeats, respectively. 4% of the population have 3, 5, 6, 8 or 10 repeats, but no humans are known to

have 0, 1 or 9 repeats. Furthermore, there are known to be at least 21 different types of these repeat units which may differ among individuals, although the first and the last repeat unit is the same for everybody (Lichter 1992, 1993).

1.4. GC and HPLC Analysis

In several of the studies in this thesis gas chromatography was used to determine antidepressant drug concentrations in brain and/or liver tissue. By definition, gas chromatography (GC) is a technique used to separate and quantify a mixture of compounds based upon the way in which these compounds partition themselves between a stationary and a mobile phase (Coutts *et al.*, 1985; Baker *et al.*, 1982).

The GC apparatus consists of several components, including an injector, an oven containing the column, a detector, an amplifier and a data recorder (integrator). The stationary phase is a non-volatile liquid, which is coated on an inert solid support, while the mobile phase is an inert gas. A solution of the compounds to be separated is injected into the injector where it is immediately vaporized and carried through the column by the inert carrier (mobile) gas. The rate at which each compound elutes from the column depends on its affinity for the stationary phase. As each compound elutes from the column, it is detected in the order in which it elutes and, after amplification of the detector's response (an electrical signal), is displayed as a peak by the integrator. The interval between the time of injection of the sample and the time at which the apex of the peak appears on the integrator is referred to as the retention time of that compound. Under specific conditions, the retention time is a characteristic property of and, thus, a means of identifying a compound.

Several detectors are available for GC analysis. These include the flame

ionization detector, thermal conductivity detector, mass-spectrometric detector, nitrogen-phosphorus detector (NPD) and the electron-capture detector (ECD). In this thesis, both the NPD and the ECD were used.

The NPD is a detector which is very sensitive to compounds containing nitrogen (N) or phosphorus (P). It consists of a heated chamber (containing a rubidium salt) into which a mixture of the effluent from the GC column, hydrogen and air is fed. Once in the chamber, the mixture is heated and a low-temperature plasma is produced which emits a minute electrical current proportional to the quantity of N- or P-containing compound present (Baker *et al.*, 1982). The current is then amplified and recorded as a peak on the integrator. Detection of N- and P-containing compounds is sensitive to approximately 5 and 200 pg, respectively.

The ECD is the most sensitive and selective detector (Burtis *et al.*, 1987) since it only detects compounds that are capable of capturing electrons and as little as 1 pg of the compound can be detected (Baker *et al.*, 1982). The ECD contains a radioactive source (^{63}Ni) which emits β -particles. These particles collide with molecules of the carrier gas, (e.g. 95% argon/5% methane), and produce slow electrons which collect at electrodes to yield a small standing current. When an electron-capturing compound elutes from the GC column, it reduces this current. This change in the current is subsequently amplified and recorded as a peak on the integrator.

In some of the experiments discussed in this thesis, HPLC was used for the analysis of levels of biogenic amines and their acidic metabolites. Liquid

chromatography consists of a process whereby a mixture of compounds is separated into its components by passing a liquid (mobile) phase containing the mixture of analytes through a stationary phase contained in the chromatographic column. An HPLC apparatus is usually comprised of the following six components: solvent reservoir, pump, injector, chromatographic column, detector and data recorder (Burtis *et al.*, 1987). The pump is used to drive the mobile phase from the reservoir to the injector, and then from the injector through the column and detector.

There are two ways in which the pump in an HPLC can operate. With the *isocratic mode* the composition of the mobile phase does not change throughout the chromatographic run. In this case, the mobile phase can be a single solvent or premixed from a variety of different solvents and then delivered from a single reservoir. With the *gradient mode* the mobile phase composition changes throughout the chromatographic run.

HPLC is generally classified into four different types based on the physicochemical processes which affect the interaction of the analytes with the mobile and stationary phases: these include adsorption, partition, ion exchange and exclusion (Bowers, 1989). Partition chromatography involves the separation of compounds based on the distribution of the solute between two immiscible liquids, one of which is the stationary phase and the other being the mobile phase. In normal-phase partition chromatography, the stationary phase is a polar solvent and the mobile phase is nonpolar. In reversed-phase partition chromatography, which was used in the HPLC analyses described in this thesis, the stationary phase is less

polar than the mobile phase.

Various detectors are available for HPLC analysis, the selectivity of which depends on its sensitivity to different compounds. The different types of detectors include ultraviolet and visible photometers, fluorescence detectors and electrochemical (EC) detectors (Burtis *et al.*, 1987; Bowers, 1989). The EC detector was used in the HPLC experiments described in this thesis. The EC detector operates by applying a potential across two electrodes which are situated within the mobile phase (Hagan, 1994). At a specific potential, a compound containing functional groups which are capable of being oxidized or reduced will undergo a reduction-oxidation reaction at the electrode surface.

There are two types of EC detectors which are referred to as coulometric and amperometric. The coulometric detector supplies the largest detector response for a given sample concentration (Bowers, 1989). Amperometric detectors, such as the one used for the experiments discussed in this thesis, have a smaller cell surface than coulometric detectors. In the amperometric cell, the effluent flows past an electrode to which a voltage has been applied. The molecules of the analyte in question can either accept electrons and be reduced or give up electrons and be oxidized. The net movement of electrons results in a flow of current which is proportional to the concentration of the analyte.

1.5. Thesis Objectives and Summary

The overall objective of this thesis was to explore the role of DA in mediating the effects of different classes of antidepressant drugs. At the time of those studies there were no known ligands which were selective for DA presynaptic autoreceptors, thus ruling out the possibility of directly measuring presynaptic autoreceptor changes by radiolabelling procedures. Therefore, the effects of chronic antidepressant treatment on the behavioural responses to low doses of the DA receptor agonists, apomorphine and quinpirole, which are believed to be selective for presynaptic DA autoreceptors, were investigated. The changes in the functional responses to these DA receptor agonists allowed for the indirect assessment of changes occurring at the DA receptor level.

This area of research has previously focussed on the effects of chronically administered TCAs on the locomotor-suppressant effects of low doses of DA receptor agonists. Results of several studies (Serra *et al.*, 1979; Antelman and Chiodo, 1981; Arnt *et al.*, 1984; Volosin *et al.*, 1991) have reported an attenuation of the locomotor-suppressant effect of low doses of DA receptor agonists. Nomikos *et al.* (1991) proposed, based on their observation that chronic administration of DMI did not attenuate the autoreceptor-mediated decrease in interstitial DA induced by a low dose of apomorphine, that the modulation of DA receptor agonist-induced effects by chronic antidepressant treatment may be due to a decrease in the DA uptake site density.

Therefore, the initial objective of this thesis was to observe the effects of the

chronically administered TCAs, DMI and CMI, as well as the MAO inhibitor, PLZ, on the locomotor-suppressant response to the DA receptor agonist apomorphine at a dose believed to be selective for presynaptic DA autoreceptors. In addition, the effects of these chronically administered antidepressant drugs on the density of DA uptake sites were assessed.

CMI, a potent 5-HT uptake inhibitor, was included because it is used in the treatment of obsessive-compulsive disorder, the symptoms of which are similar to the stereotypy which presents following the stimulation of DA systems. In view of this, it was of interest to see how CMI might mediate its effects through the DA system.

The lack of attenuation by CMI on the behavioural responses to the low-dose DA receptor agonists, apomorphine and quinpirole, discussed in chapters 3 and 4, respectively, prompted a study with FLU, a selective inhibitor of 5-HT re-uptake, in order to confirm a proposal, based on the findings with CMI, that SSRIs may not mediate their effects by decreasing presynaptic DA autoreceptor function. The role of DA in mediating the effects of TMP, an antidepressant whose mechanism of action is unknown, was also studied.

It was also of interest to investigate how these different antidepressants may affect the D₁-like versus the D₂-like DA receptor systems independently. Thus, the effects of chronic administration of these same antidepressant drugs, in addition to the selective, MAO-A inhibitor, CLG, on the behavioural responses to a D₁-like DA receptor agonist, SKF 38393, and a D₂-like DA receptor agonist, quinpirole, which is more selective for D₃ than for D₂ DA receptors, were assessed.

The attenuation of quinpirole-induced locomotor suppression by CLG suggested that selective inhibition of MAO-A may be a sufficient condition for mediating the effects of MAO inhibitors. In view of the emerging interest in the use of reversible, selective (type-A) MAO inhibitors, it was of interest to determine whether these new MAO inhibitors mediate their effects in the same manner as the irreversible MAO inhibitors. Therefore, the effects of chronic administration of the reversible, selective MAO-A inhibitors (MOC and BROF) on DA autoreceptor-mediated functional responses were compared with those of the irreversible MAO inhibitors PLZ (nonselective) and CLG (selective, type-A). This study, which used oral administration versus the s.c. administration of drugs in my previous experiments, yielded behavioural results discrepant from those observed following s.c. administration of PLZ despite comparable levels of MAO inhibition. A subsequent study was then conducted in which the effects of a range of doses of PLZ (chronic, p.o.) on the locomotor-suppressant response to apomorphine were assessed. PEA levels were also measured to determine if this trace amine is an important mediator of PLZ's effects on DA autoreceptor-mediated functional responses.

The effects of a range of concentrations of the DA receptor agonist, apomorphine, on K⁺-stimulated [³H]DA release in rat nucleus accumbens prisms were investigated as the preliminary part of an experiment which was proposed to elucidate the selectivity of apomorphine and quinpirole for D₂ versus D₃ DA receptors. From this information it was hoped that studies could be carried out in order to further confirm the DA receptor subtype through which the antidepressant

drugs may be mediating their effects.

Each of these studies, several of which formed the basis for published and submitted manuscripts, are presented in the following chapters.

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CHAPTER 2

General Methods

2.1. Chemical List

Table 2. Chemicals used in the experiments described in this thesis.

Chemicals	Suppliers
acetic acid - glacial	BDH Chemicals (Toronto, Ont.)
acetic anhydride	Caledon Laboratories (Georgetown, Ont.)
apomorphine hydrochloride	Sigma
ascorbic acid	Fisher Scientific
benzylamine	Sigma
bovine serum albumin	Sigma
brofaromine	Ciba Geigy
calcium chloride	Fisher Scientific
chloroform	BDH Chemicals
clomipramine hydrochloride	Sigma
clorgyline hydrochloride	Research Biochemicals Inc.
desmethyylimipramine hydrochloride	Sigma
diethyl ether	BDH Chemicals
DCC (dicyclohexylcarbodiimide)	Sigma
DEHPA (di(2-ethylhexyl)phosphoric acid)	Sigma
DMSO (dimethyl sulfoxide)	BDH Chemicals
³ H-dopamine	DuPont - NEN Products
EDTA	Sigma
ethyl acetate	BDH Chemicals
(±)-fluoxetine hydrochloride	Eli Lilly & Co.
folin - phenol reagent	Sigma
glycerol	Sigma

glucose	Fisher Scientific
gum tragacanth	Fisher Scientific
hydrochloric acid	Fisher Scientific
7-OH-DPAT [R(+)-7-hydroxy-2-(di- <i>n</i> -propylamino)tetralin]	Research Biochemicals
hydroxytryptamine binoxalate 5-[2- ¹⁴ C]	DuPont - NEN Products
isopentane	BDH Chemicals
magnesium sulphate	Fisher Scientific
maprotiline	Ciba-Geigy (Summit, NJ)
methoxyflurane	MTC Pharmaceuticals
moclobemide	Hofmann-La Roche
nialamide	Sigma
norleucine	BDH Chemicals
PFBS (pentafluorobenzenesulfonyl chloride)	Sigma
PfPh-OH (pentafluorophenol)	Sigma
perchloric acid	Fisher Scientific
phenelzine sulfate	Sigma
2-phenylethylamine hydrochloride	Sigma
[¹⁴ C]-2-phenylethylamine	DuPont - NEN Products
potassium carbonate anhydrous	Fisher Scientific
potassium chloride	Fisher Scientific
quinpirole hydrochloride	Research Biochemicals Inc.
scintillation fluid (Ready Safe™)	Beckman Instruments Inc. (Edmonton, AB)

SKF 38393 [2-(dipropylamino)-5,6,7,8-tetrahydro-7-naphthalenol]	Research Biochemicals Inc.
sodium bicarbonate	Fisher Scientific
sodium carbonate anhydrous	Fisher Scientific
sodium chloride	Fisher Scientific
sodium hydroxide	Fisher Scientific
sodium phosphate, dibasic, anhydrous	Fisher Scientific
sodium phosphate, monobasic	Fisher Scientific
toluene, distilled in glass	BDH Chemicals
toluene, reagent grade	BDH Chemicals
trimipramine maleate	Sigma
tris(hydroxymethyl)aminomethane (TRIS)	Fisher Scientific

2.2. Instrumentation and Apparatus

2.2.1. Locomotor Activity Monitoring System

The activity monitoring system (Acadia Instruments Ltd., Saskatoon, Saskatchewan, Canada) consisted of six 17" x 17" x 12" plexiglass tests cages each placed in a 12 x 12 beam infra-red grid system (Acadia Infra-red Grid Model 17-12 with vertical sensors). The test cages' sensors were interfaced with a microcomputer system (Acadia 6502 Data Gatherer) for data logging and temporal analysis of activity counts.

2.2.2. Filtration

A Brandel Cell Harvester equipped with Whatman GF/C filters was used for the filtration step in the [^3H] GBR 12935 uptake binding site assays.

2.2.3. Centrifuges

A Sorvall GLC-2B or Sorvall GLC-1 General Laboratory Centrifuge (Dupont Instruments) was employed for low-speed, small volume centrifugations. Higher speed and/or larger volume centrifugations were carried out in a Damon-IEC B-20 refrigerated high-speed centrifuge or a Beckman L755 vacuum refrigerated ultracentrifuge.

2.2.4. Ultraviolet Spectrophotometer

A Pye Unicam SP 1700 ultraviolet spectrophotometer was used for

determination of protein concentrations in receptor binding homogenates.

2.2.5. Tissue Homogenizer

A combination of a TRI-RS63C variable speed laboratory motor with a Teflon™ glass pestle and a glass grinding tube was used for homogenizing tissue samples.

2.2.6. Shaker-Mixer

Two types of vortex-shakers were used: Ika-Vibrax VXR2 Shaker (Janke and Kunkel Instruments) and a thermolyne Maxi Mix™ vortex mixer (Sybron/Thermolyne Instruments).

2.2.7. Weighing Balances

A Mettler AE 160 electronic balance was used for weighing chemicals and biological samples.

2.2.8. Glassware Cleaning

All glassware was rinsed with tap water and washed with biodegradable Sparkleen (Fisher Scientific Co.) solution. Further washing was accomplished with a dishwasher (Miele Electronic 6715, Germany). For test tubes, an additional cleaning step was added; test tubes were sonicated (Ultra-sonic cleaner, Mettler Electronics) in a solution of Decon 75 concentrate (BDH Chemicals) before the dishwasher wash. All glassware was then air-dried in a mechanical convection

oven (Model 28, Precision Scientific Group, Chicago, IL).

2.2.9. Liquid Scintillation Spectrometry

A Beckman LS 7500 liquid scintillation spectrometer coupled to a Datamex 43 printer was used for counting radioactivity in all ex vivo receptor binding, MAO inhibition, and [^3H]DA release studies.

2.2.10. Gas Chromatography

For determination of TCA (DMI, CMI) levels, a Hewlett Packard (HP) Model 5880 gas chromatograph equipped with a fused silica column (25 m x 0.32 mm) and a nitrogen phosphorus detector (NPD) linked to an HP 5880 integrator was used. The carrier gas was pure helium at a flow rate of 2 ml/min. The detector was purged with pure hydrogen (3.5 ml/min) mixed with dry air at 80 ml/min. The injection port temperature was 250°C and the detector temperature was 325°C. The initial oven temperature was 105°C which was programmed to increase at a rate of 25°C/min to a final temperature of 295°C.

Determination of FLU, NFLU, TMP and NTMP levels was performed using an HP 5890 gas chromatograph equipped with a DB5 column, a NPD, an HP 6890 autosampler and an HP 3396 integrator. The carrier gas was pure helium at a flow rate of 2 ml/min. The detector was purged with pure hydrogen (3.5ml/min) mixed with dry air at 80 ml/min. The injection port temperature was 250°C and the detector temperature was 325°C. The initial oven temperature was 105°C which was programmed to increase at a rate of 15°C/min to a final temperature of 295°C.

For 2-phenylethylamine (PEA) level determinations, an HP-5890 gas chromatograph equipped with a 5% phenylmethyl silicone column, an electron-capture detector (ECD) with a radioactive source of 15 mCi ^{63}Ni , an HP 7673A autosampler and an HP 3392A integrator was used. The carrier gas, helium was set at a flow rate of 2 ml/min. Argon-methane (95%-5%), flow rate 35 ml/min. was the make-up gas used in the detector. The injection port temperature was 200°C and the detector temperature was 325°C. The initial oven temperature was 105°C which was maintained for 5 min. This temperature was programmed to increase at a rate of 15°C/min to a final temperature of 270°C.

Determination of GABA levels was performed on an HP 5890 gas chromatograph equipped with a fused silica capillary column (25 m x 0.32 mm x 1.05 μm thickness) cross-linked 5% phenylmethylsilicone stationary phase, an ECD with a radioactive source of 15 mCi ^{63}Ni , an HP 7673A automatic sampler and an HP 3392A integrator. The carrier gas, helium, was set at a flow rate of 2 ml/min. Argon-methane (95%-5%), flow rate 35 ml/min. was the make-up gas used in the detector. The injection port temperature was 200°C and the detector temperature was 325°C. The initial oven temperature was 100°C which was maintained for 5 min. This temperature was programmed to increase at a rate of 25°C/min to 200°C. After maintaining at 200°C for 0.5 min, the temperature was then increased at a rate of 3°C/min to a final temperature of 230 °C.

2.2.11. High Pressure Liquid Chromatography (HPLC)

Levels of the biogenic amines and their acid metabolites and TRP were

determined using an HPLC system consisting of a solvent delivery system (Model 510 pump, Waters Associates, Milford, MA) coupled to an automatic sample injector (WISP, Waters model 710B). The compounds of interest were separated on a Phenomenex C18 column (4.6 mm i.d. x 250 mm, 5 μ m particle size, CA USA) coupled to a precolumn. Eluants from the column were detected by an electrochemical detector (model 460, Waters) with a glassy carbon electrode with the applied potential set at 0.88 volt vs an Ag/AgCl reference electrode. Chromatographic peaks were recorded and integrated using a model 740 integrator (Waters). The mobile phase, pumped at a flow rate of 0.8 ml/min, consisted of 55 mM sodium phosphate monobasic, 0.85 mM sodium octyl sulfate, 0.37 mM disodium EDTA and 10% v/v acetonitrile. The mobile phase was filtered through a type HA filter (0.45 μ m, Millipore) before being degassed and adjusted to a pH value of 3 using phosphoric acid.

2.2.12. Animals

Male Sprague-Dawley rats (150-250 g) were obtained from Health Sciences Laboratory Animal Services, University of Alberta. The animals were housed in plastic cages on cedar chip bedding in a room with an alternate 12h light/dark schedule (lights on 7:30 a.m.); the temperature of the room was maintained at $21 \pm 1^\circ\text{C}$. Food and water were freely available. The animal feed (Lab-Blox feed, Wayne Feed Division, Continental Grain Company, Chicago, IL, U.S.A.) was 4.0% (min) crude fat, 4.5% (max) crude fibre and 24% (min) crude protein. Procedures involving the use of rats were approved by the University of Alberta Health Sciences

Animal Welfare Committee and were conducted according to the guidelines established by the Canadian Council on Animal Care.

2.2.13. Drug Administration

For chronic s.c. drug administration, animals were randomly allocated to drug or VEH (control) treatment conditions. Each animal was deeply anesthetized with methoxyflurane, as an inhalant, and under aseptic conditions, were implanted with 2ML4 Alzet (Alza Corp., Palo Alto, CA) osmotic minipumps (mean pumping rate $2.5 \pm 0.14 \mu\text{l/h}$) in the interscapular region for s.c. drug delivery. Each pump was filled with a drug solution individually adjusted in concentration (Greenshaw, 1986) or distilled water VEH according to each animal group allocation to provide constant infusion. The incisions were closed with wound clips and after recovery the animals were placed in normal housing conditions and handled approximately every second day (to manipulate pumps and prevent necrosis). All chronic s.c. drug treatments described in this thesis were for 28 days. Doses of these drugs reported in this study are in $\text{mg kg}^{-1} \text{d}^{-1}$ of the salt of the respective drug (PLZ sulfate, DMI HCl, CMI HCl, CLG HCl) or of the free base dose of the drug (FLU HCl and TRI maleate) and control animals received distilled water or 25% DMSO respectively. The drug doses used in this thesis were chosen based on previous results in this laboratory and their use in published reports in the literature.

For chronic oral drug administration animals were randomly allocated to drug or VEH treatment conditions. A gavage tube was fitted on to a 1 ml syringe and the feeding tube was guided into the animal's mouth and directed into its esophagus.

Control animals were administered the drug VEH in this manner in order to account for any possible confounds introduced into the experiment as a result of the drug administration procedure. All chronic p.o. drug treatment described in this thesis was for 16 - 30 days. Doses of these drugs reported in this study are in $\text{mg kg}^{-1} \text{d}^{-1}$ of the free base dose of the respective drug (PLZ 10, PLZ 17, PLZ 30, MOC and BROF).

2.2.14. Locomotor Activity Testing

The effects of chronic antidepressant treatment on the behavioural responses (locomotor activity, yawning or grooming, depending on the experiment) to acute administration of the low-dose D_2 -like DA receptor agonists, (-)-apomorphine or (-)-quinpirole, or the D_1 -like DA receptor agonist, ($\pm$)-SKF 38393, was conducted on either days 21 or 22 (s.c. admin.) of antidepressant treatment or days 14 and 15 of treatment with PLZ at 17 and 30 mg kg^{-1} p.o. The appropriate behavioural responses were observed at these time points since these time intervals are believed to correspond to the latency with which brain receptor changes are thought to occur following initiation of antidepressant treatment in the clinical situation (Heninger and Charney, 1987).

On these behavioural testing days, a counterbalanced procedure equivalent to that described by Paetsch *et al.* (1993) was followed in which half of the animals in each chronic drug treatment group were randomly chosen to receive the acute drug ((-)-apomorphine, (-)-quinpirole or ($\pm$)-SKF 38393) injection on day 14 or 21 while the other half received the acute VEH injection. On the following day (15 or

22), the acute injection administrations were reversed so that each animal received the alternate injection. As a result of this procedure, each subject served as its own control. All acute injections were made in a 1 ml kg⁻¹ injection volume. The doses of apomorphine and quinpirole were chosen based on those used in previous studies to behaviourally assess changes in DA autoreceptor function.

Following administration of the acute VEH or drug injection (5min, quinpirole, SKF 38393; 15 min, apomorphine), the animals were placed, individually, in a computer-controlled infrared activity measurement system (see General Methods Section 1.6) where their locomotor activity was measured for 30 min in a quiet test environment under conditions of reduced illumination. Each movement made by the animal breaks a photobeam which is automatically recorded as a locomotor count.

2.2.15. Sample Collection and Storage

Following drug administration, animals were killed by guillotine decapitation and the brains were either rapidly removed and dissected over ice or placed immediately in isopentane, kept cold on dry ice, and then transferred to vials and kept frozen at -80°C until neurochemical analysis was performed. Liver tissue was also rapidly removed, transferred to vials and kept frozen at -80°C until neurochemical analysis was performed. In the experiments in which animals received chronic drug treatment by s.c. administration, the animals were killed on the morning of the last day of chronic drug administration. In the experiments in which animals received chronic p.o. drug treatment, rats were killed 1 h following the

morning drug administration on the last day of drug treatment.

2.2.16. MAO Activity Analysis

A modification of the procedure of Lyles and Callingham (1982) was used to determine the levels of MAO activity in the experiments described in this thesis. Brain and liver tissue were homogenized in 5 volumes of ice-cold distilled water. For brain tissue, 20 μ l aliquots of the homogenate were added to tubes kept on ice and diluted with 300 μ l of 0.2 M potassium phosphate buffer (pH 7.8) to yield a 1:16 dilution. The same procedure was followed when measuring MAO-A activity in liver tissue. When measuring liver MAO-B activity, 5 μ l aliquots of homogenate were diluted with 395 μ l of 0.2 M potassium phosphate buffer to yield a 1:80 dilution.

From this point, the procedure for both brain and liver was the same. To each tube, 50 μ l of VEH tissue homogenate (for control blanks, 50 μ l of VEH tissue homogenate and 10 μ l of 3M HCl) were added. 50 μ l of the appropriately labelled substrate was added and then each tube was flushed briefly with oxygen and rubber-stoppered immediately. [^{14} C] 5-HT and [^{14} C]PEA were used as substrates for MAO-A and MAO-B activity, respectively. Samples were incubated at 37°C for 10 min. The tubes were then placed on ice and 10 μ l of 3M HCl was added immediately to all samples, except the blanks, to terminate the reaction. 1ml of ethyl acetate:toluene (1:1 v/v water-saturated) was added to all tubes which were then briefly vortexed twice. Samples were centrifuged at 2000 x g briefly (30 sec). 700 μ l of the organic (top) layer, containing the aldehyde and acid products, was pipetted into scintillation vials. Readysafe™ scintillation fluid (4.5 ml) was then

added and the radioactivity level in each tube was counted for 3 min using liquid scintillation spectrometry.

Drug Level Analysis:

2.2.17. Analysis of DMI, CMI and DMCMI in Rat Brain

For determination of brain levels of DMI, CMI and DMCMI, a modification of the procedure of Drebit *et al.* (1988) was used. Rat brain tissue (excluding nucleus accumbens and caudate nucleus) was homogenized in 5 volumes of ice-cold distilled water, and 2 ml of the total homogenate was used for analysis. Maprotiline (internal standard) at a concentration of 1000 ng was added to each homogenate sample. Samples were then basified with solid sodium carbonate followed by the addition of ethyl acetate (4 ml). The compounds of interest were extracted into the ethyl acetate layer by shaking (5 min) and centrifugation (5 min). The organic (top) layer was transferred to a clean set of tubes and then taken to dryness under a stream of nitrogen gas. The residues were then reconstituted in 2 ml of distilled water and acetylated by adding acetic anhydride under basic conditions using the procedure of Martin and Baker (1977). The acetylated maprotiline, DMI and DMCMI and underivatized CMI were then extracted into ethyl acetate (4 ml) by shaking (5 min) and centrifugation (5 min). The compounds of interest (organic top layer) were transferred to a clean set of tubes and then taken to dryness in a Savant (Savant Speedvac SC110 with a refrigerated condensation trap) evaporator. The residues were reconstituted in 300 μ l of toluene, and a 1 μ l aliquot was injected on a gas chromatograph equipped with a NPD (Drebit *et al.*, 1988) which is described in the

General Methods section 1.6. A standard curve was run concurrently with the tissue samples in this and all other GC assays.

2.2.18. Analysis of FLU, NFLU, TMP and NTMP in Rat Brain

For determination of brain levels of FLU, NFLU, TMP and NTMP in rat brain the procedure followed was the same as that used for the determination of DMI, CMI and DMCMI except for the following modifications. Rat brain tissue used for analysis consisted of a half brain since the other half was used for HPLC analysis of biogenic amine levels. Maprotiline was added at a concentration of 2000 ng to each sample which was then basified with 250 μ l of 25% K_2CO_3 (potassium carbonate).

2.2.19. Analysis of PEA in Rat Brain and Liver

A modification of the GC assay developed by Baker *et al.* (1986) was used for the analysis of PEA in rat brain and liver. Brains or liver were homogenized in 5 volumes of ice-cold 0.1 M perchloric acid ($HClO_4$) containing EDTA. The homogenates were centrifuged for 10 (brain) or 20 (liver) min at 12,000 x g at 0-4°C. A 3 ml aliquot of the supernatant was used for the analysis and 125 ng of benzylamine was added as an internal standard to all samples. The samples were basified by the addition of solid potassium bicarbonate and the precipitate removed by a brief centrifugation. The resultant samples were then transferred to a clean set of tubes. A 10% (300 μ l) volume of sodium phosphate buffer (0.25M, pH=7.8) was added and the samples were extracted by shaking (5 min) with 5 ml of chloroform

containing the liquid ion-pairing reagent di(2-ethylhexyl)phosphoric acid (DEHPA, 2.5%, v/v). Following a 5 min centrifugation, the top (aqueous) layer was removed by aspiration and the remaining chloroform layer was transferred to a clean set of tubes. The chloroform layer was then shaken vigorously for 5 min with 4 ml of 0.5 M HCl. After centrifuging for 5 min, the top (aqueous) layer was transferred to clean tubes and basified with solid sodium bicarbonate. A solution (4ml) of ethyl acetate-acetonitrile-pentafluorobenzenesulfonyl chloride (PFBS) (9:1:0.01) was added to each tube. The samples were shaken (15 min) and centrifuged (5 min). The top (organic) layer was transferred to a set of clean tubes and taken to dryness in a Savant (Savant Speedvac SC110 with a refrigerated condensation trap) evaporator. Each residue was then reconstituted in 200 μ l of toluene, and a 2 μ l aliquot was injected on a gas chromatograph equipped with an ECD as described in the General Methods section 2.2.10.

2.2.20. Analysis of GABA Levels

To determine brain GABA levels, a procedure developed by Wong *et al.* (1990) was used. Half of the brain was homogenized in 5 volumes of ice-cold 0.1 N perchloric acid containing 0.05 mM ascorbic acid and 10.0 mg % EDTA. The homogenates were then centrifuged at 10,000 x g for 15 min at 4°C. A 25 μ l aliquot of the supernatant was then used for analysis of GABA levels. Norleucine (250 ng) was added as an internal standard to the supernatant. The mixture was basified with 1 ml of 2.5% w/v potassium carbonate followed by the addition of 1ml isobutylchloroformate solution (5 μ l/ml toluene:acetonitrile 9:1 v/v). After shaking

for 10 min and centrifuging for 2 min at 2500 x g, the organic phase (top layer) was aspirated and discarded. 1.5 ml sodium phosphate buffer (2M, pH 6) and 25 µl 6N HCl were then added and the samples vortexed briefly. The following were then added in sequential fashion: 2.5 ml chloroform, 200 µl dicyclohexylcarbodiimide (DCC) solution (5 µl/ml chloroform) and 200 µl pentafluorophenol (PfpPh-OH). This mixture was then vortexed for 15 min. Following a brief (5 min) centrifugation, the aqueous (top) layer was then aspirated and discarded. The bottom chloroform layer was then decanted into a clean tube and evaporated to dryness under a stream of nitrogen gas. The residue was reconstituted in 300 µl of toluene and washed briefly with 500 µl of distilled water. This mixture was vortexed for less than 5 sec. After a rapid centrifugation, the organic (top) layer was removed and an aliquot of this layer was used for GC analysis as described in the General Methods section 2.2.10. For all GC analyses described in this thesis, a standard (calibration) curve was prepared in parallel with each assay to allow for the quantitation of the compound of interest in brain homogenate or supernatant or liver homogenate samples. The standard curve was constructed by adding known, varying amounts of authentic standards of the compounds of interest and a fixed amount (same amount as added to the tissue homogenate or supernatant) of internal standard to a series of tubes containing homogenate or supernatant of drug-naive brains and carrying these solutions through the assay in parallel with the solutions containing sample tissue.

2.2.21. Analysis of Biogenic Amines and their Acid Metabolites

To determine levels of NA, DA, DOPAC, 5-HT, 5-HIAA and TRP, rat brains,

after partial thawing, were homogenized in 5 volumes of ice-cold distilled water. To a 450 μ l aliquot of the homogenate, 50 μ l of perchloric acid containing 10 mg % EDTA and 0.5 mM ascorbic acid, was added. The homogenate was centrifuged at 12,000 x g for 1.5 min to remove the protein precipitate. A 150 μ l aliquot of the supernatant was then injected on to the HPLC apparatus described in the General Methods section 2.2.10. A standard (calibration) curve of known concentrations of the biogenic amines and metabolites were run in parallel to each group of samples. The standard curves were linear, with correlation coefficients of $r^2 > 0.99$ being obtained routinely. The peak height ratios of amines or acid metabolites to internal standard in the samples were compared to those in the standard curve to determine the amount of amine or acid metabolite in each sample.

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CHAPTER 3

Chronic Antidepressant Drug Treatment Attenuates Motor-Suppressant Effects of Apomorphine Without Changing [³H]-GBR 12935 Binding

3.1. Introduction

Early analysis of antidepressant drug action focused on brain NA and 5-HT but it is now clear that antidepressants differ widely in their effects. Some antidepressant drugs [eg. nomifensine and bupropion] appear to have relatively selective actions on the DA system (for review Jimerson, 1987; Osman and Potter, 1991). Additionally, there is clinical evidence for DA dysfunction in depression (for review, Davis, 1989; Mann and Kapur, 1995). Low concentrations of HVA, a DA metabolite, have been found in the CSF of suicidal patients (Oreland *et al.*, 1981; Traskman *et al.*, 1981; Lindberg *et al.*, 1985), and CSF levels of HVA have been reported to correlate negatively with scores obtained on the Hamilton Depression Rating Scale (Roy *et al.*, 1985; Peabody *et al.*, 1987). The issue of DA abnormalities in psychotic depression has also been addressed, with high plasma HVA levels being reported in such patients (Devanand *et al.*, 1985; Mazure *et al.*, 1987).

Preclinical studies with rodents have demonstrated marked effects of antidepressants on aspects of brain DA systems. Enhancement of behavioural responses to systemic administration of direct and indirect DA receptor agonists, such as apomorphine and (+)-amphetamine, respectively, has generally been observed following chronic administration of tricyclic (imipramine and DMI) and of MAO inhibitor (pargyline and CLG) antidepressants (Maj *et al.*, 1979, 1984; Spyraiki and Fibiger, 1981; Martin-Iverson *et al.*, 1983; Campbell *et al.*, 1985; De Ceballos *et al.*, 1985; Serra *et al.*, 1990). In contrast, behavioural responses to low doses of

apomorphine, a treatment believed to be selective for D₂ DA autoreceptors, are usually inhibited by chronic antidepressant treatment (Serra *et al.*, 1979; Arnt *et al.*, 1984; Volosin *et al.*, 1991). Although these studies have in general been limited to TCAs, the findings suggest that a decrease in DA autoreceptors, which are negatively coupled to DA synthesis, may be responsible for the behavioural sensitization of the DA system seen after repeated antidepressant treatment. Electrophysiological and neurochemical data also generally indicate that DA autoreceptor activity may be reduced following chronic treatment with antidepressants such as imipramine or amitriptyline (Serra *et al.*, 1979; Antelman and Chiodo, 1981; Lee and Tang, 1982; Nielsen, 1986). However, chronic administration of DMI has been reported to have no effect on interstitial DA concentrations in the nucleus accumbens and not to attenuate the autoreceptor-mediated decrease in interstitial DA induced by low-dose apomorphine (Nomikos *et al.*, 1991). This latter finding has led to the suggestion that the potentiation of behavioural effects of DA receptor agonists by antidepressant treatment may be due to a decrease in DA uptake sites, but at the time of conducting this thesis research there were no data concerning such changes in uptake site density (Nomikos *et al.*, 1991).

After chronic treatment, most antidepressant drugs appear to increase 5-HT neurotransmission as measured by electrophysiological methods (Blier *et al.*, 1987; Blier and de Montigny, 1994). 5-HT receptors are present in the VTA where there are cell bodies of DA-containing neurons and in the nucleus accumbens where

there are terminals of DA-containing neurons (Pazos *et al.*, 1991; Jacobs and Asmitia, 1992). It is possible, therefore, that enhanced 5-HT neurotransmission after chronic antidepressant treatment may play a role in the enhancement of behaviours induced by DA receptor agonists. Indeed, chronic treatment with citalopram, a selective 5-HT uptake blocker [$IC_{50}=1.8$ nM, $K_i=1.3$ nM] (Hyttel, 1982; Richelson and Pfenning, 1993), is reported to result in an attenuation of apomorphine-induced hypolocomotion and an enhancement of amphetamine-induced behaviours (Arnt *et al.*, 1984). This dose of citalopram, however, may have locomotor-stimulant effects (Arnt *et al.*, 1984), indicating that the observed attenuation of apomorphine-induced hypolocomotion may possibly be secondary to this stimulant effect of citalopram. This does not, however, rule out the possible interaction of 5-HT and DA in the increased response to DA receptor agonists observed after chronic antidepressant treatment.

The present study examined the effects of chronic administration of two TCAs, DMI and CMI, and of the MAO inhibitor PLZ on the locomotor-suppressant effects of a low dose of apomorphine. In addition, the effects of these treatments on [^{3} H]-GBR 12935 binding to the DA uptake site were also examined. CMI was included because it predominantly acts to inhibit 5-HT uptake (Hyttel, 1982) and is preferentially active in the treatment of obsessive-compulsive disorder, the features of which parallel to some extent the stereotypy seen following stimulation of DA systems (Zohar and Insel, 1987; Goodman *et al.*, 1990).

3.2. Materials and Methods

3.2.1. Animals and Surgery

Adult male Sprague-Dawley rats weighing 320-400g at the time of surgery were used. Animals were randomly allocated to one of four drug treatment groups ($n=14-15$) and were implanted with 2ML4 Alzet osmotic minipumps, as described in the General Methods section 2.2.13, for s.c. drug delivery. The chronic drug-treatment groups received either DMI HCl, PLZ sulfate, or CMI HCl at a dose of $10 \text{ mg kg}^{-1} \text{ d}^{-1}$ and control animals received distilled water. Doses are expressed as the salts. After 28 days of continuous drug infusion, rats were killed by guillotine decapitation and their brains rapidly removed. The caudate nucleus and nucleus accumbens were quickly dissected out over ice and then stored at -80°C until the time of analysis. The rest of the brain tissue was also immediately frozen over dry ice and stored at -80°C until used for estimates of drug levels and MAO activity.

3.2.2. Drug Levels and MAO Activity

For determination of brain levels of DMI, CMI and DMCM, a modification of the procedure of Drebit *et al.* (1988) was used as described in the General Methods section 2.2.17.

MAO activity was determined radiochemically using a modification of the procedure of Lyles and Callingham (1982) as described in the General Methods section 2.2.16.

3.2.3. Behavioural Testing

Between days 21 and 22 of chronic drug or VEH administration, the effects on spontaneous locomotor activity of (-)-apomorphine HCl (0.05 mg kg⁻¹) and of the VEH (0.1% ascorbic acid dissolved in 0.9% saline) were assessed as explained in the General Methods section 2.2.14. Apomorphine or VEH were injected s.c. with an injection volume of 1 ml/kg 15 min prior to testing.

3.2.4. DA Uptake Site Binding

Saturation binding analysis of the selective DA uptake site antagonist [³H]-GBR 12935 (specific activity 44.2 Ci/mmol) was used to quantify the density and affinity of DA uptake sites in the rat caudate nucleus using an adaptation of the methods of Janowsky *et al.* (1986) and Morissette *et al.* (1990). Rat caudate nucleus tissue was homogenized in 8 ml of ice-cold 50 mM Tris buffer (pH=7.4) containing 120 mM NaCl and further diluted by adding an additional 8 ml of this buffer. The homogenate was centrifuged at 40,000 g for 10 min. The pellet was resuspended in 16 ml of the above buffer and centrifuged again at 40,000 g for 10 min. The resulting tissue pellet was then resuspended in buffer. Saturation analyses were performed in duplicate, in Fisherbrand™ disposable polystyrene tubes (Fisher Scientific, Canada), in a total volume of 1 ml of buffer containing 100 µl of tissue suspension and [³H]-GBR 12935 (0.05 to 2.0 nM). To define non-specific binding, GBR 12909 (1 µM) was used. The plastic tubes were incubated for 60 min at 4°C and the contents were rapidly filtered using a Brandel cell harvester and washed with a large excess of ice-cold incubation buffer. Filters had

been presoaked, for 2 hours, in a 2 g/l solution of poly(ethylenimine) to reduce non-specific binding. After filtration, the filters were placed in 5 ml of Ready Safe™ liquid scintillation cocktail (Beckman, Mississauga, Canada) and the amount of radioactivity measured; counting efficiency was approximately 52%. Protein determinations were carried out using the method of Lowry *et al.* (1951) and the final Bmax estimates, derived from computer analysis with EBDA and LIGAND (McPherson, 1987), were expressed in fmol/mg protein.

Single point binding analysis using [³H]-GBR 12935 was carried out using tissue from the nucleus accumbens. The procedure was analogous to the one described above, with nucleus accumbens tissue (n=6-7) pooled from two rats, homogenized/resuspended in 8 ml of buffer and 100 µl of this homogenate was incubated with 0.5 nM [³H]-GBR 12935 in a total volume of 1 ml of buffer. Protein determinations were carried out (as above) to accurately quantitate the amount of binding for between-sample comparisons and statistical analysis.

3.2.5. Statistics

The data were subjected to analysis of variance as described by Winer (1971), with a criterion of $p < 0.05$. For comparisons between means following a significant F test, the Newman-Keuls multiple comparison test was used with a criterion of $p < 0.05$.

3.3. Results

3.3.1. Drug Levels and MAO Activity

Brain levels of DMI were 1582 ± 210 ng/g tissue (5939 ± 788 nM) in animals treated with DMI. In animals treated with CMI, brain levels of CMI were 678 ± 60 ng/g tissue (2153 ± 190 nM) and brain levels of DMCM, the secondary amine derived from the metabolism of CMI, were below detectable limits of the assay (i.e. < 75 ng/g tissue). Type A and B MAO were inhibited by $93.1 \pm 1.0\%$ and $94.7 \pm 0.3\%$, respectively, in brain tissue from animals treated with PLZ.

3.3.2. Behavioural Testing

The mean locomotor activity of the VEH group receiving acute 0.1% ascorbic acid VEH injections was 3094 ± 165 counts/30 min (i.e. photocell beam breaks). Chronic treatment with antidepressant drugs alone did not result in any statistically significant difference in this measure of baseline activity assessed on days 21/22 [$F(3,55)=1.13$, $p>0.05$]. The mean ($\pm$ SEM) locomotor activity (counts/30 min) of the DMI, CMI and PLZ treatment groups receiving acute VEH injections was 2229 ± 164 , 2844 ± 172 and 2459 ± 133 counts/30 min, respectively. Statistical analysis indicated that there was a significant effect of apomorphine alone on locomotor activity [$F(1,55)=134.71$, $p<0.05$] as well as a significant interaction between chronic antidepressant treatment and the acute apomorphine challenge [$F(3,55)=5.54$, $p<0.05$]. The effects of low-dose apomorphine on spontaneous locomotor activity for each treatment group are shown in Figure 5. Acute apomorphine injections

resulted in a decrease in spontaneous locomotor behavior in the chronic VEH treatment group to $39 \pm 5\%$ of that seen after acute saline injections. Chronic treatment with DMI and PLZ significantly attenuated the response to acute apomorphine administration. DMI and PLZ reversed the apomorphine-induced suppression of activity to $64 \pm 6\%$ and $66 \pm 15\%$, respectively, of that seen after acute ascorbate/saline challenge. CMI, on the other hand, did not attenuate the behavioural response to apomorphine. In fact, locomotor activity was suppressed to 37% of that observed following acute saline injections. Although there were no significant effects of antidepressants on baseline activity, the activity of the chronic antidepressant-treated group tended to be lower than that of the chronic VEH-treated group. The effects of the antidepressants on the apomorphine response are not attributable to changes in baseline activity as both CMI- and PLZ-treated groups had similar average baseline activities and only the PLZ-treated group exhibited an attenuated apomorphine response.

3.3.3. DA Uptake Site Binding

Specific binding of [^3H]-GBR 12935 to rat caudate nucleus DA uptake sites was saturable and a single population of high affinity binding sites was indicated as Scatchard plots were linear. In control animals the average affinity (K_d) of these sites was 0.886 ± 0.079 nM and the average density (B_{max}) of DA uptake sites was 2804 fmol/mg protein. These values are in close agreement with previously published values (Janowsky *et al.*, 1986; Morissette *et al.*, 1990). Antidepressants had no significant effect on either the K_d [$F(3,32)=0.13$, $p>0.05$] or B_{max}

[$F(3,32)=0.41$, $p>0.05$] of DA uptake sites in the caudate nucleus, as seen in Table 3. In rat nucleus accumbens tissue, the specific binding of 0.5 nM [^3H]-GBR 12935 to DA uptake sites was also unaffected by chronic antidepressant treatment, as shown in Figure 6.

3.4. Discussion

The enhancement of behavioural responses to systemic administration of DA receptor agonists observed following chronic antidepressant treatment has been well documented (Maj *et al.*, 1979, 1984; Spyraiki and Fibiger, 1981; Martin-Iverson *et al.*, 1983; Campbell *et al.*, 1985; De Ceballos *et al.*, 1985), although there are few data available describing effects of antidepressants on the density of DA receptors. Chronic treatment with DMI decreases the density of D_1 -like DA receptors (Klimek and Maj, 1990), whereas chronic PLZ treatment actually decreases the density of both D_1 -like and D_2 -like DA receptors (Paetsch and Greenshaw, 1992). It is possible that increased DA neurotransmission due to a decrease in D_2 -like DA autoreceptors may be responsible for the overall enhancement of behavioural responses to DA receptor agonists. Previous reports have demonstrated that chronic TCA (imipramine and DMI) treatment attenuates the behavioural effects of a low dose of apomorphine which is selective for D_2 -like DA autoreceptors (Serra *et al.*, 1979; Volosin *et al.*, 1991). The present study confirms that chronic DMI treatment does attenuate apomorphine-induced hypolocomotion in rats. This is in contrast to another study (Spyraiki and Fibiger, 1981) which found no effect of DMI

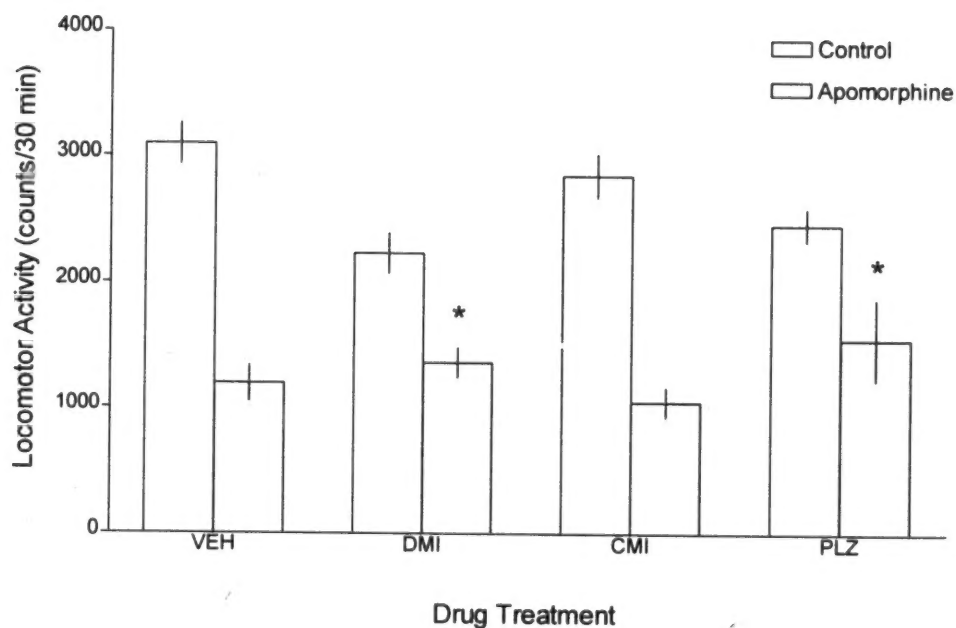


Figure 5: Effects of acute apomorphine HCl on locomotor activity of rats following chronic treatment with VEH, DMI, CMI and PLZ. Data shown are means $\pm$ S.E.M. (n=14-15). DMI and PLZ attenuated apomorphine-induced locomotor suppression. *Effects were significant at $p < 0.05$, ANOVA.

Drug Treatment	K _d (nM)	B _{max} (fmol/mg protein)
VEH	0.886 ± 0.079	2804 ± 147
DMI	0.818 ± 0.081	3189 ± 160
CMI	0.853 ± 0.090	2812 ± 462
PLZ	0.825 ± 0.093	2982 ± 208

Table 3: Effects of chronic (28 d) antidepressant drug treatment on [³H]-GBR 12935 binding to the DA uptake site in rat caudate nucleus. Data are means ± S.E.M. (n=8-10 per group).

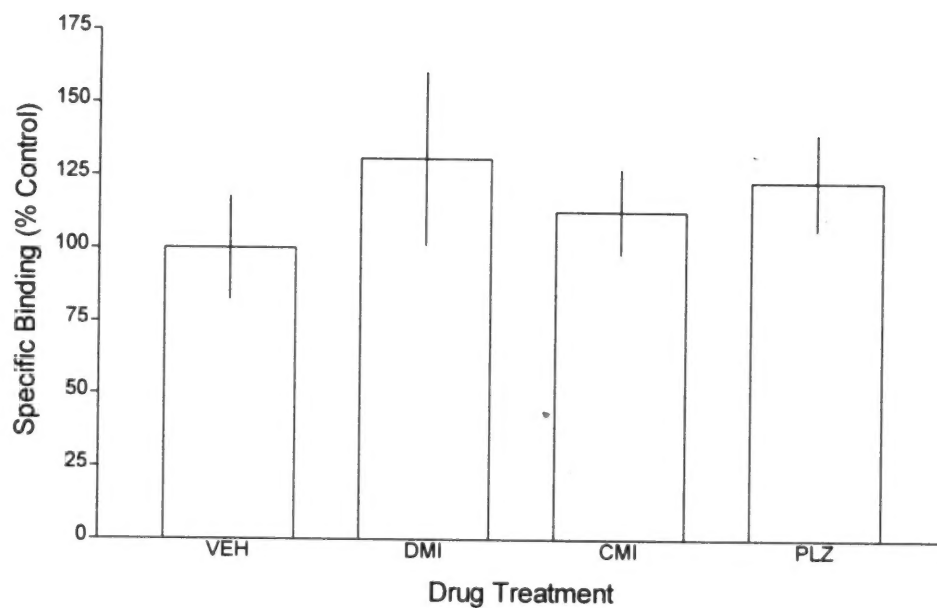


Figure 6: Lack of effect of chronic antidepressant treatments on the amount of specific [^3H]-GBR 12935 (0.5 nM) binding to the DA uptake site in the nucleus accumbens (n=6-7: tissue from two rats was pooled for each determination).

on the locomotor-suppressant response induced by low-dose apomorphine. The reason for the discrepancy between the present study and that of Spyraiki and Fibiger (1981) is unknown. It is possible that the present discrepancy is attributable to the difference in drug administration protocol between the studies: Spyraiki and Fibiger administered 5 mg kg⁻¹ DMI twice per day i.p. for 14 days and in the present study, DMI was administered 10 mg kg⁻¹ DMI per day s.c. via minipump, with apomorphine testing on days 21 and 22. Another possibility is the difference in sample size used in each study. The previous study (Spyraiki and Fibiger, 1981) used a smaller sample size (N=10) compared with that used in our study (N=15). Since results from the other study did reveal a tendency of chronically administered DMI to attenuate apomorphine-induced hypolocomotion, it is possible that the increased power due to a larger sample size may have resulted in statistically significant effects (Winer, 1971). In the present study, the range of antidepressants tested has been extended by showing that chronic treatment with the MAO inhibitor PLZ also attenuates the motor-suppressant effects of low-dose apomorphine. The TCA, CMI, which is potent at blocking 5-HT uptake [$IC_{50}=1.5$ nM, $K_i=5.4$ nM] (Hyttel, 1982; Richelson and Pfenning, 1984), was detected in the brain of rats treated with CMI in the present study. The desmethylated product of CMI, which is less potent at inhibiting 5-HT uptake sites [$IC_{50}=41$ nM] (Hyttel, 1982) but has relatively potent effects on NA uptake [$IC_{50}=0.46$ nM] (Hyttel, 1982), was not detectable in these same rats. The failure to detect DMCM following chronic administration of CMI contrasts strongly with our previous results indicating the formation of large quantities of DMI following chronic administration of imipramine whereas our DMI

levels are consistent with previous results from this laboratory (McManus *et al.*, 1991). Fung *et al.* (1994) have found that CMI was much less extensively demethylated in the rat than was imipramine, suggesting that, in the rat, CMI is relatively selective as an inhibitor of 5-HT uptake. The results of the present experiment indicate that chronic CMI treatment, at a dose that does result in functional down-regulation of β -adrenoceptors (Mousseau *et al.*, 1992), does not result in the attenuation of apomorphine-induced motor suppression. This finding suggests that chronic inhibition of 5-HT uptake may not result in a functional down-regulation of presynaptic DA receptors. Nevertheless, although the literature generally indicates a relative selectivity of CMI for 5-HT uptake, particularly in the absence of detectable quantities of DMCM, we did not measure the specificity of 5-HT uptake blocking effects of this treatment in the present study. Therefore, the question of whether or not chronic and selective 5-HT uptake inhibition will result in changes in DA receptor responsiveness remains open and will be the focus of the studies in chapter 6 which include the SSRI, FLU.

Although the attenuation of apomorphine-induced hypolocomotion appears to indicate that D₂-like DA autoreceptors may be down-regulated by chronic DMI and PLZ treatment, a previous study by Spyraiki and Fibiger (1981) did not support this finding. Furthermore, chronic DMI administration has been reported to have no effect on the decrease in interstitial DA concentration in the nucleus accumbens induced by low-dose apomorphine (Nomikos *et al.*, 1991). It was suggested, therefore, that antidepressants may act by decreasing DA uptake sites (Nomikos

et al., 1991). The present study investigated this possibility by measuring the affinity and density of [^{3} H]-GBR 12935 binding to DA uptake sites. The K_d and B_{max} values observed for [^{3} H]-GBR 12935 binding in the caudate nucleus are consistent with previously reported values (Morissette *et al.*, 1990). There were no apparent changes in either DA uptake site affinity or density in the caudate nucleus. More importantly, in the nucleus accumbens there was no significant change in the amount of binding to DA uptake sites. It appears, therefore, that changes in behavioural responses to DA receptor agonists observed after chronic antidepressant treatment are not due to a decrease in DA uptake sites.

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CHAPTER 4

Behavioural Response to SKF 38393 and Quinpirole Following Chronic Antidepressant Treatment

4.1. Introduction

Several studies indicate a role for brain DA in antidepressant drug action (Spyraki and Fibiger, 1981; Martin-Iverson *et al.*, 1983; Maj *et al.*, 1989b; Serra *et al.*, 1991; Brown and Gershon, 1993; Gessa, 1994). A variety of electrophysiological and neuropharmacological data indicates that chronic antidepressant treatment may decrease DA autoreceptor-mediated responses (Chiodo and Antelman, 1980a,b; Antelman and Chiodo, 1981; Nielsen, 1986). In addition, chronic antidepressant treatment may attenuate the suppression of locomotor activity induced by low doses of D₂-like DA receptor agonists (Serra *et al.*, 1979; Maj *et al.*, 1989a; Allison *et al.*, 1993). These observations form the basis of the hypothesis that a decrease in the number or function of presynaptic DA receptors may play an important role in mediating the effects of chronic antidepressant treatment (Serra *et al.*, 1979; Chiodo and Antelman, 1980a,b; Antelman and Chiodo, 1981; Nielsen, 1986). A decrease in uptake site density has also been proposed as a mechanism for antidepressant effects which have generally been attributed to changes in presynaptic DA receptors (Nomikos *et al.*, 1991). This seems unlikely as chronic antidepressant treatment may not alter the affinity or density of [³H]-GBR 12935 DA uptake sites (Allison *et al.*, 1993).

Data from several studies indicate that behavioural responses to high doses of D₂-like DA receptor agonists may be potentiated following chronic antidepressant treatment (Spyraki and Fibiger, 1981; Serra *et al.*, 1990a). This potentiation may be attributable to the development of presynaptic DA autoreceptor subsensitivity

and/or postsynaptic DA receptor supersensitivity (Serra *et al.*, 1979, 1990a; Spyraiki and Fibiger, 1981; Gessa, 1994). Studies of chronic antidepressant effects on behavioural responses to D₁-like DA receptor agonists have so far not yielded any clear results (Chagraoui *et al.*, 1990; Maj, 1990; Serra *et al.*, 1990a; Wachtel *et al.*, 1992).

The effects of chronic administration of MAO inhibitor antidepressants have received very little attention in this context. Maj (1990) has reported that chronic administration of the selective, reversible type-A MAO inhibitor MOC may result in decreased sedative effects of a low dose of apomorphine and may induce a potentiation of the stimulant effects of a high dose of this drug. Nevertheless, neither BROF nor MOC appear to alter the stimulant effects of (+)-amphetamine (Maj, 1990). In contrast to the effects of the irreversible MAO inhibitor PLZ (Paetsch and Greenshaw, 1992), BROF and MOC do not appear to alter the density or affinity of D₁- or D₂-like DA receptors (Klimek and Maj, 1990; Klimek *et al.*, 1990).

In the present study, the effects of chronic tricyclic and irreversible MAO inhibitor antidepressant treatment on the behavioural responses to both DA D₁-like (SKF 38393) and low-dose D₂-like DA (quinpirole: D₂/D₃) [Sokoloff *et al.*, 1992] receptor agonists were compared. The antidepressants were chosen as compounds that decrease D₁- and D₂-like DA receptor density (PLZ: Paetsch and Greenshaw, 1992) or D₁-like DA receptor density (DMI: Klimek and Nielsen, 1987; Klimek and Maj, 1990; Paetsch and Greenshaw, 1992) following their chronic administration. CLG was selected as a MAO-A inhibitor for comparison with the non-selective

inhibitory actions of PLZ on MAO. CMI was also included in this study to more clearly assess its effects on DA receptor function in this context; in a previous study, chronic administration of CMI did not result in any changes in the locomotor-suppressant effects of the DA receptor agonist apomorphine (Allison *et al.*, 1993).

4.2. Materials and Methods

4.2.1. Animals and Surgery

Male Sprague-Dawley rats weighing between 250 and 310 g at the time of surgery were used. Animals were randomly allocated to one of five drug treatment groups (n=14-15) that received either DMI HCl, CMI HCl, PLZ sulphate, CLG HCl or the distilled water VEH. Antidepressant drug doses were administered at salt doses of 10 mg kg⁻¹ (DMI, CMI, and PLZ) or 1 mg kg⁻¹ (CLG). Animals were implanted with 2ML4 Alzet osmotic mini-pumps, as described in the General Methods section 2.2.13., for s.c. drug delivery. On the 28th day of drug administration, the rats were killed by guillotine decapitation. The brains were immediately removed and stored at -80°C until required for analysis of MAO activity and determination of brain drug levels. These measurements were taken to ensure the efficacy of drug administration.

4.2.2. Drug Levels and MAO Activity

Brain tissue concentrations of DMI, CMI and DMCM, the demethylated metabolite of CMI were determined by gas chromatography using a modification of

the procedure of Drebit *et al.* (1988) as described in the General Methods section 2.2.17.

MAO activity was determined radiochemically using a modification of the procedure of Lyles and Callingham (1982) as described in the General Methods section 2.2.16.

4.2.3. Behavioural Testing

On days 21 and 22 of chronic drug or VEH administration, the effects of either (-)-quinpirole HCl (0.05 mg kg^{-1}), ($\pm$)-SKF 38393 HCl (10 mg kg^{-1}) or distilled water on spontaneous locomotor activity, grooming and yawning were measured.

Doses of quinpirole and SKF 38393 are expressed as the free base. Spontaneous locomotor activity was measured using a counterbalanced procedure as explained in the General Methods section 2.2.14. Quinpirole, SKF 38393 or distilled water was injected subcutaneously in a volume of 1 ml kg^{-1} 5 min prior to each behavioural test.

During the behavioural testing period, yawning and grooming were measured under blind conditions by two observers using a modification of the procedure of Molloy and Waddington (1987). Each animal was observed for 1 min at 5 min intervals, yielding 5 min of observation over each 30 min test. The chosen doses of quinpirole (0.05 mg kg^{-1}) and SKF 38393 (10 mg kg^{-1}) were found in other studies to reliably elicit yawning and to increase grooming, respectively (Jackson *et al.*, 1989a; Longoni *et al.*, 1987; Molloy and Waddington, 1987; Serra *et al.*, 1987).

Prior to this experiment, a pilot study was conducted with separate groups of subjects to confirm that these doses were effective. Since grooming is part of the neophobic behavioural repertoire elicited by a novel environment (Molloy and Waddington, 1987), all rats were habituated to the test environment for 15 min per day for the 6 days prior to behavioural test days.

4.2.4. Statistics

Effects of drug treatment on locomotor activity and time spent grooming were analysed using analysis of variance (ANOVA). In the case of a significant F test, a Newman-Keuls multiple comparison test was used to determine significant differences between means ($\alpha=0.05$) (Winer, 1971). A Kruskal-Wallis 1-way ANOVA was used to assess the effects of drug treatment on yawning and grooming. For these measures, the significance of differences between means was then assessed by Mann-Whitney U tests using a Bonferroni correction for the α level (Krauth, 1988).

4.3. Results

4.3.1. Drug Levels and MAO Activity

Concentrations of DMI and CMI in the brain tissue of rats treated with these drugs were 1487.5 ± 215.8 (5584 ± 810 nM) and 844.5 ± 94.7 (2682 ± 300 nM) ng/g tissue, respectively. Brain levels of DMCM, the metabolite of CMI, if present, were below the detectable limits of the assay (i.e. <75 ng/g tissue). In the brain tissue of

rats treated with CLG, inhibition of MAO-A and -B was $88.1 \pm 1.7\%$ and $9.8 \pm 2.0\%$, respectively. In PLZ-treated rats, inhibition of MAO-A and -B was $87.7 \pm 1.2\%$ and $93.9 \pm 0.3\%$, respectively. These data are in accord with the results of previous experiments in this laboratory and confirm that the chronic drug administration procedure was effective.

4.3.2. Behavioural Testing

4.3.2.1 Behaviours Observed Following Quinpirole Probe

Following the acute VEH challenge, locomotor counts per 30 min (mean $\pm$ S.E.M.) were as follows: VEH, 2345 ± 276 ; DMI, 1380 ± 244 ; CMI, 1544 ± 189 ; PLZ, 1869 ± 240 ; CLG, 1309 ± 135 . There was an effect of antidepressant treatment [$F(4,70)=2.62$, $p<0.05$], with DMI and CLG significantly decreasing locomotor activity. The acute challenge with quinpirole induced a decrease in spontaneous locomotor activity [$F(1,70)=224.20$, $p<0.05$]: the activity of chronic VEH-treated rats was reduced to $28 \pm 3\%$ of that seen following the distilled water injection (see Figure 7). The locomotor-suppressant effect of quinpirole was attenuated [$F(4,70)=5.75$, $p<0.05$] following chronic treatment with each of the antidepressants tested except CMI, as illustrated in Figure 7. The scattergram of Figure 8 illustrates the distribution of yawning in each of the treatment groups following administration of quinpirole. Yawning was not observed in any of the animals following the acute distilled water injection. Following the administration of quinpirole, yawning was observed in VEH-, DMI-, and CMI-treated animals but not in the PLZ- and CLG-treated animals [$H=34.18$, $df=4$]. Statistical analysis of time spent grooming

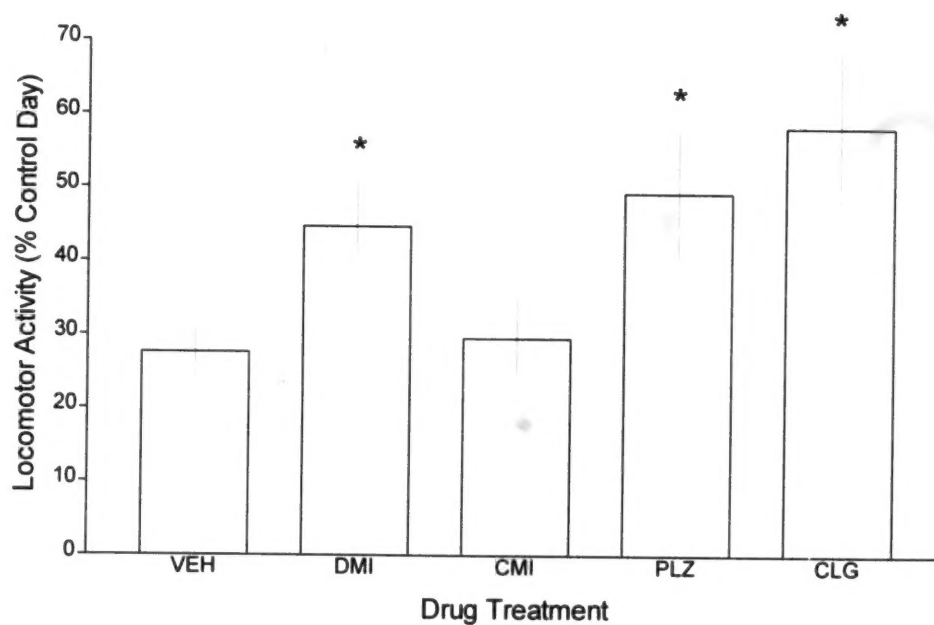


Figure 7. Effects of quinpirole on spontaneous locomotor activity of rats following chronic VEH or antidepressant treatment. Data shown are means $\pm$ S.E.M. (n=15). DMI, PLZ and CLG attenuated quinpirole-induced locomotor suppression. *Effects were significant at $p < 0.05$, relative to VEH.

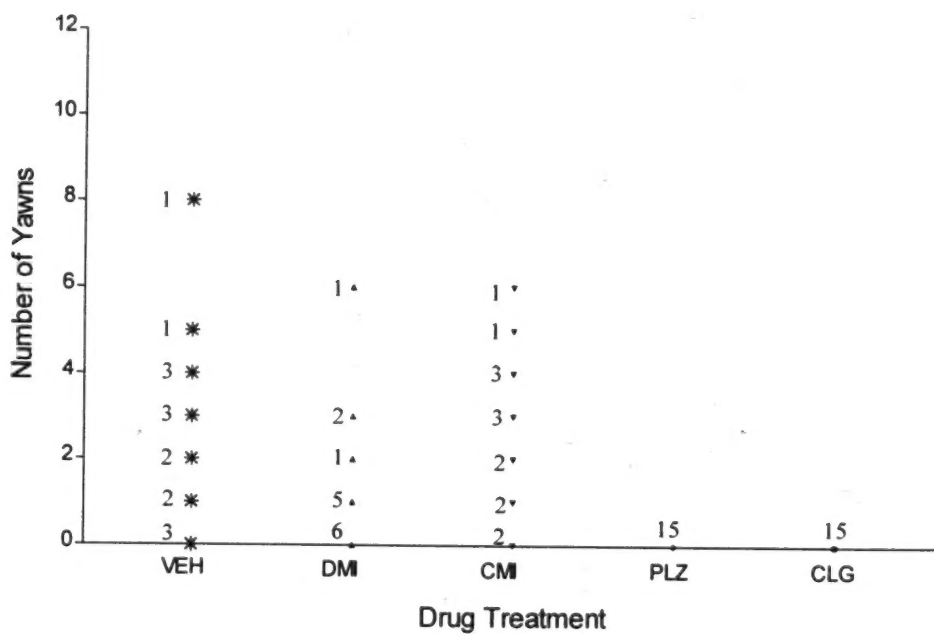


Figure 8. Effects of quinpirole on the number of yawns observed in rats following chronic VEH or antidepressant treatment. Numbers beside asterisks or triangles indicate the number of rats exhibiting the specified number of yawns ($p < 0.05$, relative to VEH). Data shown are means $\pm$ S.E.M. ($n=15$).

revealed no significant effect of either antidepressant treatment [$F(4,70)=0.55$, $p>0.05$] or quinpirole [$F(1,70)=1.28$, $p>0.05$]. There was also no effect of antidepressant treatment [$H=3.65$, $df=4$] or quinpirole [$H=-5.46$, $df=4$] on the number of grooming bouts.

4.3.2.2 Behaviours observed following SKF 38393 probe

Following the acute VEH challenge, locomotor counts per 30 min (mean $\pm$ S.E.M.) were as follows: VEH, 1701 ± 268 ; DMI, 1282 ± 185 ; CMI, 1388 ± 231 ; PLZ, 1779 ± 217 ; CLG, 1825 ± 287 . There was no statistically significant effect of antidepressant treatment on locomotor activity [$F(4,69)=1.47$, $p>0.05$], although the activity of the DMI and CMI groups appear to be decreased relative to controls. SKF 38393 increased locomotor activity [$F(1,69)=19.18$, $p<0.05$] (Figure 9) and time spent grooming [$F(4,69)=108.08$, $p<0.05$] (Figure 10). In chronic VEH-treated rats, this increase in locomotor activity was $126 \pm 14\%$ of the control level. Chronic antidepressant treatment had no effect on SKF 38393-induced hyperactivity [$F(4,69)=1.94$, $p>0.05$] or on the SKF 38393-induced increase in grooming [$F(4,69)=1.60$, $p>0.05$]. There was no significant effect of antidepressant treatment [$H=0.79$, $df=4$] or of the SKF 38393 probe [$H=6.08$, $df=4$] on the number of grooming bouts (data not shown). Yawning was not exhibited by any of the rats following either the distilled water or SKF 38393 injection.

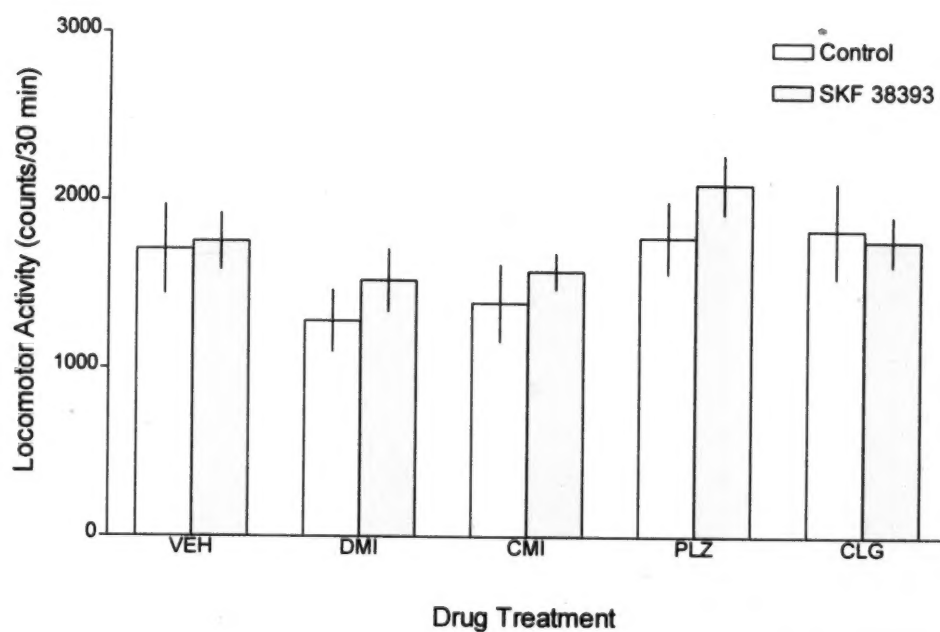


Figure 9. Effects of SKF 38393 on spontaneous locomotor activity of rats following chronic VEH or antidepressant treatment. Data shown are means $\pm$ S.E.M. ($n=14-15$). Chronic antidepressant treatment had no effect on the increased locomotor activity induced by SKF 38393.

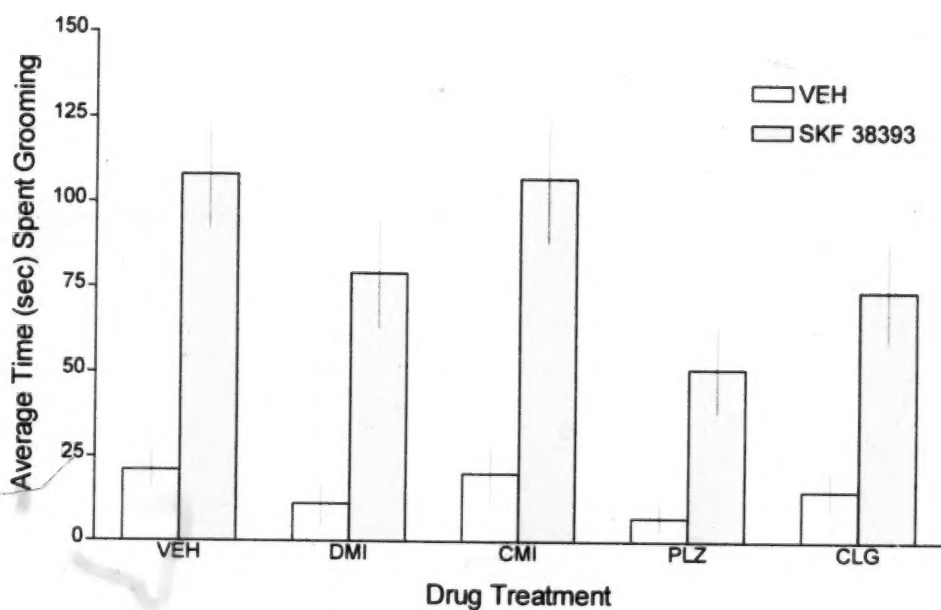


Figure 10. Effects of SKF 38393 on time spent grooming in rats following chronic VEH or antidepressant treatment. Data shown are means $\pm$ S.E.M. (n=14-15). Chronic antidepressant treatment had no effect on the increase in time spent grooming induced by SKF 38393.

4.4. Discussion

In the present experiment, decreased sensitivity to quinpirole was observed following chronic administration of the TCA, DMI, and the MAO inhibitors PLZ and CLG. These results are consistent with a previous report of decreased locomotor-suppressant effects of apomorphine at 0.05 mg kg^{-1} following chronic treatment with DMI or PLZ (Allison *et al.*, 1993).

Serra *et al.* (1990a,b) observed an attenuation of the locomotor-suppressant effects of quinpirole at 0.05 mg kg^{-1} , but not at 0.01 mg kg^{-1} , following chronic administration of the TCA imipramine. They suggested that this dose-related effect indicates that chronic antidepressant treatment results in a postsynaptic DA receptor supersensitivity rather than a presynaptic autoreceptor subsensitivity. Serra *et al.* (1990a,b) argued that the decreased locomotor suppressant effects of quinpirole were attributable to a shift to the left in the dose-response curve for postsynaptic DA activation. The observations that the densities of D_2 -like DA receptors may be reduced following chronic administration of irreversible MAO inhibitors (Paetsch and Greenshaw, 1992), and that the locomotor-suppressant effects of quinpirole (this study) and of apomorphine (Allison *et al.*, 1993) are decreased following chronic MAO inhibition are inconsistent with this postsynaptic supersensitivity hypothesis.

It seems likely that a decreased density of D_2 -like DA receptors resulting from chronic treatment with MAO inhibitors (Paetsch and Greenshaw, 1992) contributed to the absence of quinpirole-induced yawning in PLZ- and CLG-treated rats. In the

present study, the TCAs did not alter quinpirole-induced yawning. This is consistent with the observation that chronic TCA (DMI) treatment does not result in a decrease in the density of D₂-like DA receptors (Klimek and Nielsen, 1987; Klimek and Maj, 1990; Paetsch and Greenshaw, 1992).

In this study, the control value for locomotor activity was increased following the administration of SKF 38393. The time spent grooming was also increased, but, the number of grooming bouts was not altered, in accord with the results of Wachtel *et al.* (1992). The lack of an interaction between chronic antidepressant treatment and the effects of SKF 38393 in the present study is in agreement with the results of Serra *et al.* (1990a). Maj *et al.* (1989a) have demonstrated that a SKF 38393-induced increase in grooming was attenuated at 2 but not 48 hours following acute or chronic antidepressant administration. It is likely that this effect may be the result of a direct interaction between the effects of SKF 38393 and of primary antidepressant treatment effects, rather than emergent receptor changes. Chagraoui *et al.* (1990) reported that 2 days following chronic administration of amineptine or DMI, there was a decrease in SKF 38393-induced locomotor activity; nevertheless, there were no changes in SKF 38393-induced grooming. Decreases in D₁-like DA receptor density have been measured following chronic administration of tricyclic or MAO inhibitor antidepressants (De Montis *et al.*, 1990a,b; Paetsch and Greenshaw, 1992). These biochemical changes do not correspond to changes in the present behavioural responses to a D₁-like DA receptor agonist.

The results of this study with respect to CLG suggest that selective inhibition

of MAO-A may be a sufficient condition for changes in D₂-like DA receptor agonist responses. This is potentially an important result in view of the emergent interest in selective and reversible MAO-A inhibitor antidepressants (Callingham, 1993). This effect of CLG is in agreement with the observation of decreased apomorphine-induced sedation following chronic administration of the reversible MAO-A inhibitor MOC (Maj, 1990), although the degree and selectivity of MAO inhibition was not reported in that study.

In the present study and that of Allison *et al.* (1993), the locomotor suppression induced by a low dose of quinpirole and of apomorphine was not attenuated by chronic administration of CMI, a potent 5-HT uptake inhibitor ($IC_{50}=1.5$ nM, $K_i=5.4$ nM) (Hyttel, 1982; Richelson and Pfenning). CMI is not as selective for 5-HT uptake blockade as compounds such as FLU. Nevertheless, in the present study, the demethylated metabolite of CMI, DMCM, which is less selective for inhibiting 5-HT uptake ($IC_{50}=41$ nM) (Hyttel, 1982) but more potent at inhibiting NA uptake ($IC_{50}=0.46$ nM) (Hyttel, 1982), was not detected in the brain tissue of animals chronically treated with CMI. The absence of DMCM in the brain tissue of rats treated with CMI in this and a previous study (Allison *et al.*, 1993) suggests that CMI may not be metabolized in rats as extensively as it is in humans. This is consistent with the study by Fung *et al.* (1994) which demonstrated that CMI is demethylated less extensively in rats than is imipramine. In accord with the present CMI results, 5-HT uptake blockers such as citalopram, ifoxetine, FLU and zimelidine fail to affect either the stimulant effects of amphetamine or yawning

induced by apomorphine (Martin-Iverson *et al.*, 1983; Delini-Stula and Hunn, 1990; but see Pucilowski and Overstreet, 1993). The present CMI results and the observations described above suggest that, in contrast to MAO inhibitors and DMI, antidepressants which are potent inhibitors of 5-HT uptake may not mediate their effects by decreasing presynaptic DA autoreceptor function.

4.5. References

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CHAPTER 5

Estimation of The *In Vivo* Potency of A Reversible MAO Inhibitor

5.1. Introduction

Due to the reversible nature of MOC and BROF, two selective MAO-A inhibitors which will be discussed further in the next chapter, it was necessary to demonstrate the *ex vivo* efficacy of these MAO inhibitors as well as to confirm that their ability to inhibit MAO-A was comparable to that of the irreversible MAO inhibitors. Thus, a protection experiment was carried out with MOC according to the procedure of Green (1984) which proposes that the degree to which a reversible MAO inhibitor can protect against inhibition by an irreversible MAO inhibitor is an estimation of the *in vivo* MAO inhibition by a reversible MAO inhibitor. Green (1984) states that there are several factors which may affect the interpretation of the results of a MAO inhibition assay when reversible MAO inhibitors are assayed compared with irreversible MAO inhibitors. Because the reversible MAO inhibitors do not form a stable covalent bond with MAO like the irreversible MAO inhibitors do, they may become dissociated from MAO while the tissue is homogenized and their concentration in the tissue homogenate may become diluted with the addition of substrate and buffer. As a result, the concentration of inhibitor may be much lower than that which was originally present in the original tissue due to the extensive dilution which occurs during the preparation of the tissue homogenate and during the subsequent addition of substrate and other reagents, such as buffer, required for the assay. Therefore, the MAO assay may not measure the degree of inhibition in the original tissue, but the degree of inhibition produced by the concentration of inhibitor which is ultimately reached in the *ex vivo* assay mixture. In other words,

the true level of inhibition by the reversible MAO inhibitors will be underestimated and the *ex vivo* measurement of MAO inhibition by reversible MAO inhibitors may be an underestimate of their *in vivo* efficacy.

5.2. Materials and Methods

5.2.1. Animals and Drug Administration

Male Sprague-Dawley rats weighing approximately 400 g were used. Rats were housed 2 per cage on a 12-hour light/dark cycle with food and water freely available. Animals were randomly allocated to one of four drug treatment groups ($n=10$) in which they were administered their respective drug treatment followed 15 minutes later by a subsequent administration of the appropriate drug. Group 1 was the control group and received the VEH followed by another VEH injection. Group 2 received MOC followed by a VEH injection. Group 3 was the "protection group" and received MOC followed by PLZ. Group 4 received PLZ followed by VEH. The VEH treatment consisted of dimethyl sulfoxide (DMSO) made up in distilled water.

All drug doses are expressed as the free base and were orally administered at a dose of 15 mg kg⁻¹ (MOC) or 4 mg kg⁻¹ (PLZ). 24 hours following the second drug administration, rats were killed and the brains rapidly removed and frozen at -80°C until required for analysis of MAO activity.

5.2.2. MAO Activity

MAO activity was determined radiochemically using a modification of the

procedure of Lyles and Callingham (1982) as described in the General Methods section 2.2.16. An acute protection experiment was carried out according to the method of Green (1984) (see section 5.2.1. above) in order to estimate the *in vivo* MAO-inhibiting potency of the reversible MAO inhibitors.

5.2.3. Statistics

The data were subjected to analysis of variance (ANOVA). In the case of a significant F test, a Newman-Keuls multiple comparisons test was carried out to determine significant differences between means ($\alpha=0.05$) (Winer, 1971).

5.3. Results

5.3.1. MAO Activity

Levels of MAO inhibition are presented as a percent of control MAO inhibition (Figure 11). In the brain tissue of rats from groups 2, 3 and 4 (see section 5.2.1. above), the inhibition of MAO-A was -2.5 ± 6.9 , 89.5 ± 4.1 , and $81.9 \pm 5.0\%$, respectively. Corresponding values of inhibition of MAO-B were -5.5 ± 7.1 , 15.5 ± 3.6 , and $65.4 \pm 2.5\%$, respectively.

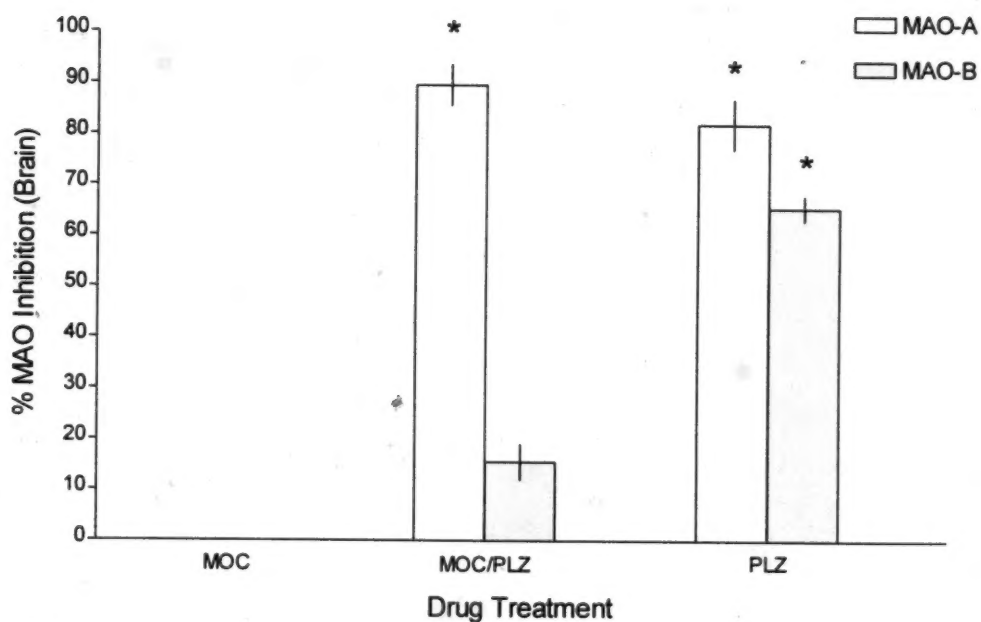


Figure 11. Estimation of the *in vivo* MAO inhibition by MOC using a protection experiment. MOC administered alone shows negligible MAO inhibition. PLZ inhibits MAO nonselectively. Levels of MAO inhibition following pretreatment (15 min) with MOC prior to PLZ indicates that MAO-A inhibition by MOC *in vivo* is at least 89.5%. Data shown are means $\pm$ S.E.M. (n=5). *Effects are significant, relative to VEH, $p < 0.05$, ANOVA.

5.4. Discussion

The results of this acute protection experiment indicate that MOC is a potent and selective MAO-A inhibitor *in vivo*. In addition to this, the MAO-A inhibition by MOC is comparable to that of the irreversible MAO inhibitor PLZ. The results obtained in the present study are consistent with those of Green (1984) which used harmaline as the reversible MAO-A inhibitor. Despite the use of a different MAO inhibitor, *ex vivo* values obtained for MAO inhibition by MOC in the present study are consistent with what Green (1984) obtained with harmaline. In the present acute protection experiment, MAO activity had recovered completely within 24 hours following administration of MOC alone (as would be expected with a reversible MAO inhibitor). MAO activity was still inhibited to a considerable extent 24 hours after administration of the irreversible inhibitor PLZ.

In the chronic study discussed in the following chapter (6), *ex vivo* MAO activities were determined for the reversible MAO inhibitors, MOC and BROF, and the irreversible MAO inhibitors, PLZ and CLG. Even though a protection experiment was not conducted in that study, the values obtained for MAO-A inhibition by the reversible and irreversible MAO inhibitors were comparable to one another and to the values obtained in this protection experiment; such findings indicate that there is not a marked dilution effect with MOC and BROF. Other studies (Urichuk *et al.*, 1994; Allison *et al.*, 1995) have either measured MAO inhibition by MOC, BROF or PLZ 6 hours following drug administration on day 10 or MAO inhibition by BROF or PLZ at 1 hour after drug administration on day 4 (Holt *et al.*, 1994). In each of these

studies, a protection experiment was not carried out and the degree of MAO-A inhibition by the reversible MAO inhibitors, MOC and BROF, was comparable to that obtained with the irreversible MAO inhibitor, PLZ. Furthermore, the values obtained for MAO inhibition by the reversible inhibitors in these studies were consistent with those obtained in the chronic study discussed in the following chapter (6).

In the chronic study discussed in the following chapter, the high level of MAO inhibition by MOC and BROF may have been due to the ability of these two reversible MAO inhibitors to behave as mechanism-based, enzyme activated MAO inhibitors (Amrein *et al.*, 1989; Da Prada *et al.*, 1989; Waldmeier *et al.*, 1994). In a mechanism-based, enzyme-activated reaction, the activity of the MAO inhibitor increases as the duration of interaction with the enzyme increases. It has been suggested that the enzyme-inhibitor complex may undergo a conformational change, resulting in a tighter association between the enzyme and inhibitor (Amrein *et al.*, 1989; Cesura *et al.*, 1992). In the case of MOC, there is an increased accumulation of this drug following chronic administration which may be due to the ability of MOC to inhibit its own metabolism via one of its metabolites (Mayersohn and Guentert, 1995). Although MOC concentrations were not measured in the present study, results obtained in brain tissue by Urichuk (1997) indicate that at the dose and time interval used here and for the chronic study discussed in chapter 6, the amounts of MOC and BROF present in the brain tissue would be sufficient to inhibit MAO even after dilution.

5.5. References

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Chapter 6

Differential Functional Effects of MAO Inhibitors

On DA Autoreceptors

6.1. Introduction

Following chronic antidepressant treatment, an attenuation of the locomotor-suppressant effects of low doses of DA receptor agonists has been reported (Maj, 1990; Serra *et al.*, 1990; Allison *et al.*, 1993, 1995). Since low doses of DA receptor agonists are believed to be selective for presynaptic DA autoreceptors, these findings suggest that chronic administration of antidepressant drugs may result in the functional down-regulation of presynaptic DA autoreceptors. A decrease in DA uptake sites has also been proposed as a possible mechanism for mediating the effects of antidepressant treatment (Nomikos *et al.*, 1991). This seems unlikely, however, as chronic antidepressant treatment has not been shown to alter the binding of [³H]GBR 12935 to DA uptake sites (Allison *et al.*, 1993).

Studies investigating the effects of chronic antidepressant treatment on DA autoreceptor-mediated responses initially focussed on the TCAs (Spyraki and Fibiger, 1981; Martin-Iverson *et al.*, 1983; Klimek and Maj, 1989; Serra *et al.*, 1990). The observation that a functional down-regulation of presynaptic DA autoreceptors may be a significant mechanism of antidepressant drug action has been extended to the MAO inhibitors (Maj, 1990; Allison *et al.*, 1993, 1995). Maj (1990) reported that chronic treatment with the reversible, selective type-A MAO inhibitor MOC reduced the locomotor-suppressant effects of a low dose of the DA receptor agonist apomorphine and increased the stimulant effect of a high dose of this drug. In this study, neither MOC nor BROF, however, had any effect on the locomotor-stimulant effects of (+)-amphetamine. Following repeated administration of reversible MAO

inhibitors there is apparently no change in the density or affinity of D₁ and D₂ DA receptors (Klimek *et al.*, 1990), although such changes in receptor density have been demonstrated following chronic treatment with the irreversible MAO inhibitor PLZ (Paetsch and Greenshaw, 1992).

It was previously found that chronic subcutaneous administration of PLZ attenuates the locomotor-suppressant effect of low doses of apomorphine and quinpirole (Allison *et al.*, 1993, 1995). It was also demonstrated that CLG, which is an irreversible, selective MAO-A inhibitor, attenuates the locomotor-suppressant effects of quinpirole. This result suggested that inhibition of MAO-A may be a sufficient condition for the development of functional down-regulation of DA autoreceptors.

The present study investigated the effects of chronic oral treatment with reversible, selective MAO-A inhibitors on the functional down-regulation of DA autoreceptors. The measure of DA autoreceptor activation was the locomotor-suppressant effect of a low dose of apomorphine, as in previous studies (Allison *et al.*, 1993, 1995). The effects of MOC and BROF (reversible, selective type-A) and of PLZ and CLG (irreversible, non-selective and selective type-A, respectively) MAO inhibitors were investigated.

In experiment 1 of the present study, the chronic oral administration of 10 mg kg⁻¹ PLZ did not attenuate DA autoreceptor-mediated locomotor suppression as it did in previous experiments involving subcutaneous administration (Allison *et al.*, 1993, 1994) despite comparable levels of MAO inhibition. It has been proposed

that increased levels of PEA, rather than inhibition of MAO *per se* may be important in the mechanism of action of PLZ (Paetsch *et al.*, 1993). A difference in PEA levels has been observed following a range of subcutaneous doses of PLZ concomitant with different functional responses to the β -adrenoceptor agonist, salbutamol despite comparable levels of MAO inhibition (Paetsch *et al.*, 1993).

In the second part of the present study, the effects on the locomotor-suppressant effects of low-dose apomorphine by a range of doses of PLZ were measured. Brain and liver PEA levels were also measured *ex vivo* in an attempt to determine if there is a relationship between PEA concentrations and the functional down-regulation of DA autoreceptors. In addition, brain levels of GABA and biogenic amines and their acid metabolites were measured.

6.2. Materials and Methods

6.2.1. Animals and Surgery

In experiment 1, male Sprague-Dawley rats weighing between 200-270 g on the first day of drug administration were used. Animals were randomly allocated to one of four drug treatment groups (n=15) and received either VEH, MOC, BROF HCl, CLG HCl or PLZ sulphate. VEH animals received 0.05% gum tragacanth made up in distilled water. All drug doses are expressed as the free base and were orally administered at a dose of 15 mg kg⁻¹ twice daily (MOC and BROF) or 1 mg kg⁻¹ and 10 mg kg⁻¹ once daily (CLG and PLZ, respectively). The VEH and drug solutions were administered orally in a volume of 0.8ml at 8:30 a.m. and 4:30 p.m.

A second administration of the VEH was delivered to VEH, CLG and PLZ rats to account for any possible effects of twice-daily administration.

On the 30th day of administration, rats were killed as described in the General Methods section 2.2.15 and the liver and brains (excluding cortex and hippocampi) rapidly removed frozen at -80°C until required for analysis of MAO (brain and liver) and PEA levels (liver).

In the second experiment, rats ($n=11-15$) received either the VEH (0.05% gum tragacanth) or PLZ at 17 and 30 mg kg^{-1} p.o. Since several rats receiving PLZ at these higher doses died, the study was terminated prematurely. Thus, this experiment was carried out for 16 rather than 30 days. On the 16th day of administration, rats were killed and the liver and brains were rapidly removed, placed into isopentane cooled on dry ice, transferred to vials and frozen at -80°C until required for analysis of PEA, biogenic amine and GABA levels and MAO activity.

6.2.2. PEA Levels and MAO Activity

MAO activity was determined radiochemically using a modification of the procedure of Lyles and Callingham (1982) as described in the General Methods section 2.2.16 to confirm the efficacy of drug administration. PEA levels were measured in brain (experiment 2) and liver (experiment 1 and 2) tissue using a modification of the method of Baker *et al.* (1986) as described in the General Methods section 2.2.19.

6.2.3. Levels of GABA, Biogenic Amines and Acid Metabolites

In experiment 2, brain levels of GABA and of the biogenic amines DA and 5-HT and their metabolites, HVA, DOPAC and 5-HIAA as well as NA and TRP were measured using the methodology and apparatus as described in the General Methods sections 2.2.19 and 2.2.20.

6.2.4. Behavioural Testing

On days 21 and 22 of chronic drug or VEH administration in the first experiment, the effects of low-dose apomorphine HCl (0.05 mg kg^{-1}) or the 0.1% ascorbic acid (dissolved in 0.9% saline) VEH on spontaneous locomotor activity were observed. Locomotor activity was measured using a counterbalanced procedure equivalent to that described by Paetsch *et al.* (1993) and explained in the General Methods section 2.2.14 was used. Acute injections were administered s.c. in a volume of 1 ml kg^{-1} 15 minutes prior to the behavioural test which lasted 30 minutes.

On both behavioural test days, animals received their morning drug administrations immediately following the completion of their behavioural assessment. Evening drug treatments were administered 8 hours later. For the second experiment, the behavioural test was carried out in the same manner as for experiment 1 except that the behavioural days were on the 14th and 15th day of the experiment.

6.2.5. Statistics

The data were subjected to analysis of variance (ANOVA). In the case of a

significant F test, a Newman-Keuls multiple comparisons test was carried out to determine significant differences between means ($\alpha=0.05$) (Winer, 1971).

6.3. Results

6.3.1. PEA Levels and MAO Activity

In experiment 1, PEA levels in liver were 2.1 ± 0.13 and 80.4 ± 12.7 ng/g of tissue in rats administered VEH or PLZ (10 mg kg^{-1}), respectively (Figure 12). In the brain tissue of rats treated with MOC, BROF, CLG or PLZ, the *ex vivo* inhibition of MAO-A was 71.0 ± 2.5 , 77.4 ± 2.8 , 81.4 ± 1.5 and $90.2 \pm 1.5\%$, respectively. The corresponding values for *ex vivo* inhibition of MAO-B were 10.5 ± 2.9 , 14.9 ± 4.1 , 18.6 ± 3.1 and $91.3 \pm 0.7\%$, respectively. In the liver tissue of rats treated with MOC, BROF, CLG and PLZ, inhibition of MAO-A was 92.6 ± 0.8 , 92.8 ± 1.0 , 70.0 ± 2.4 , and $97.7 \pm 0.4\%$, respectively. The corresponding values for *ex vivo* inhibition of MAO-B were 14.3 ± 4.5 , 0 ± 3.3 , 17.0 ± 4.2 and $80.8 \pm 1.5\%$ in liver tissue of rats treated with MOC, BROF, CLG and PLZ, respectively.

In experiment 2, PEA levels in brain were 1.6 ± 0.2 , and 41.8 ± 7.1 and 121.4 ± 27.6 ng/g of tissue in rats administered VEH or PLZ (17 and 30 mg kg^{-1}), respectively (Figure 12). Brain PEA levels were significantly increased relative to VEH following either dose of PLZ [$F(2,43) = 19.59$, $p < 0.05$]. Brain PEA levels at the 30 mg kg^{-1} dose were significantly higher than those obtained following administration of the 17 mg kg^{-1} dose. The corresponding liver concentrations of PEA were 3.2 ± 0.2 , 255.5 ± 34.7 and 585.3 ± 28.9 ng/g of tissue, respectively

(Figure 12). Liver PEA levels were significantly increased relative to VEH following both doses of PLZ, with levels in rats treated with the 30 mg kg⁻¹ dose being significantly higher than those measured in rats receiving the 17 mg kg⁻¹ dose [F(2,35) = 150.03, p<0.05]. In the brain tissue of rats treated with PLZ at 17 and 30 mg kg⁻¹, the *ex vivo* inhibition of MAO type A was 99.0 ± 0.1 and 99.0 ± 0.2%, respectively. Corresponding values for the inhibition of MAO type B were 95.9 ± 0.6 and 97.7 ± 0.2%, respectively. In the liver tissue of rats treated with PLZ at 17 and 30 mg kg⁻¹, *ex vivo* inhibition of MAO type A was 99.3 ± 0.1 and 99.7 ± 0.1%, respectively. Corresponding values for inhibition of MAO type B were 97.2 ± 0.3 and 97.3 ± 1.2%, respectively.

6.3.2. Levels of GABA, Biogenic Amines and Acid Metabolites

In experiment 2, GABA levels in brain were 338.34 ± 10.43, 506.52 ± 25.37 and 593.29 ± 28.30 µg/g of tissue in rats administered VEH or PLZ (17 and 30 mg kg⁻¹), respectively. GABA levels were significantly different from VEH at both doses of PLZ, with GABA levels at the 30 mg kg⁻¹ dose of PLZ being significantly greater than those observed at 17 mg kg⁻¹ [F(2,35) = 40.19, p<0.05].

In brain tissue of rats treated with PLZ at 17 and 30 mg kg⁻¹, levels of NA, DA, and 5-HT were significantly higher than those of VEH-treated rats (Table 4). The levels of these biogenic amines were not significantly different following the 30 versus 17 mg kg⁻¹ dose. The levels of DOPAC, HVA and 5-HIAA were significantly decreased in the brain tissue of rats treated with both doses of PLZ versus the VEH-treated rats but there was no significant difference between the two doses of PLZ.

TRP levels were unaffected by either dose.

6.3.3. Behavioural Testing

In the first experiment, following the acute VEH injection (0.1% ascorbic acid), locomotor counts/30 min (mean $\pm$ SEM) were as follows: VEH, 3822 ± 311 ; MOC, 2741 ± 249 ; BROF, 3078 ± 275 , CLG, 3081 ± 263 ; PLZ, 3104 ± 190 . Statistical analysis indicated that there was a significant effect of MOC, but not of the other antidepressants on locomotor activity [$F(4,70)=2.66$, $p<0.05$]. There was a significant effect of apomorphine on spontaneous locomotor activity [$F(1,70)=72.02$, $p<0.05$]: the activity of chronic VEH-treated rats was reduced to $62 \pm 10\%$ of that seen following the acute VEH challenge. There was, however, no significant interaction between chronic antidepressant treatment and the apomorphine probe [$F(4,70)=0.20$, $p>0.05$] (Figure 13).

In experiment 2, for chronic VEH-treated rats, locomotor counts were 2943 ± 219 counts/30 min following the acute 0.1% ascorbic acid injection (control probe). Following the acute control probe, the locomotor activity (mean $\pm$ SEM) of rats treated with PLZ at 17 and 30 mg kg⁻¹ were 2305 ± 324 and 2306 ± 179 counts/30 min, respectively. Statistical analysis indicated that there was no significant effect of antidepressant treatment alone on locomotor activity [$F(2,35)=0.08$, $p>0.05$]. There was a significant effect of apomorphine on spontaneous locomotor activity [$F(1,35)=5.52$, $p<0.05$] (Figure 14). In chronic VEH-treated rats, apomorphine reduced locomotor activity to $64 \pm 10\%$ of the control level. There was also a

	VEH (n=15)	PLZ 17 (n=12)	PLZ 30 (n=11)
DA	1054.18 ± 29.89	1349.69 ± 57.06*	1332.73 ± 35.55*
DOPAC	105.84 ± 4.35	10.11 ± 2.93*	12.00 ± 5.21*
HVA	65.60 ± 3.33	0.21 ± 0.21*	0.46 ± 0.46*
5-HT	467.89 ± 26.25	1483.64 ± 50.43*	1575.94 ± 46.04*
5-HIAA	242.61 ± 12.94	79.88 ± 6.72*	51.07 ± 3.80*
NA	531.61 ± 19.70	1083.91 ± 51.10*	1040.40 ± 51.88*
TRP	4.30 ± 0.21	4.39 ± 0.28	4.23 ± 0.29

Table 4. Levels of biogenic amines and their metabolites (ng/g ± SEM) and TRP (µg/g ± SEM) in whole brain following chronic (16 day) oral administration of VEH or PLZ (17 and 30 mg kg⁻¹). *Effects were significant at p<0.05, relative to VEH.

significant interaction between chronic antidepressant treatment and the apomorphine probe [$F(2,35)=3.34$, $p<0.05$]. The locomotor-suppressant effect of apomorphine was attenuated following chronic treatment with both doses (17 and 30 mg kg⁻¹) of PLZ, as illustrated in Figure 14.

6.4. Discussion

In view of the emergent interest in the reversible, selective type-A MAO inhibitors as a safer alternative to the irreversible MAO inhibitors as a treatment for depression, it was of interest to determine if these drugs mediate their clinical effects in a similar manner. In the first experiment of this study, it was demonstrated that in chronic VEH-treated animals, a challenge with low-dose apomorphine resulted in a significant decrease in locomotor activity. Chronic oral administration of any of the antidepressants alone, with the exception of MOC, did not alter spontaneous locomotor activity. It is not likely, however, that this effect of MOC contributed to the lack of attenuation of the behavioural response to apomorphine since this baseline effect was absent following treatment with PLZ, CLG and BROF and they still did not attenuate locomotor suppression. The absence of effects of MOC and BROF on apomorphine-induced locomotor suppression does not appear to be due to their selectivity for inhibition of MAO-A since it has previously been shown (Allison *et al.*, 1995) that s.c. administration of CLG, an irreversible, selective type-A MAO inhibitor, is able to attenuate the locomotor-suppressant effect of low-dose quinpirole. Furthermore, it seems unlikely

that the lack of effect is due to the reversible nature of these MAO inhibitors since PLZ and CLG, both irreversible MAO inhibitors, were also without effect in this study.

Maj (1990) has reported an attenuation of apomorphine-induced locomotor suppression following chronic oral treatment with MOC but no effect on the stimulant effect of (+)-amphetamine. There was, however, no measurement of the degree and selectivity of MAO inhibition in that study. Klimek *et al.* (1990) have demonstrated that there is no change in the density or affinity of D₁ and D₂ DA receptors following chronic oral treatment with MOC or BROF, whereas a decrease in the striatal density of D₁ and D₂ DA receptors has been reported following chronic subcutaneous treatment with PLZ (Paetsch and Greenshaw, 1992). This absence of change in the DA receptor population is consistent with the lack of effects of MOC and BROF on apomorphine-induced locomotor suppression observed in the present study despite marked and selective MAO-A inhibition. The discrepancy between the behavioural results of the present study and those of Maj (1990) may be due to a difference in experimental design. Maj (1990) administered the apomorphine probe and conducted the behavioural test 100 minutes after the oral antidepressant administration. In the present experiment, the antidepressant was administered following the behavioural test to ensure that there was no interaction between the acute apomorphine probe and the antidepressant.

In addition, in the present study, the locomotor-suppressant effect of apomorphine was not reduced following chronic oral administration with CLG

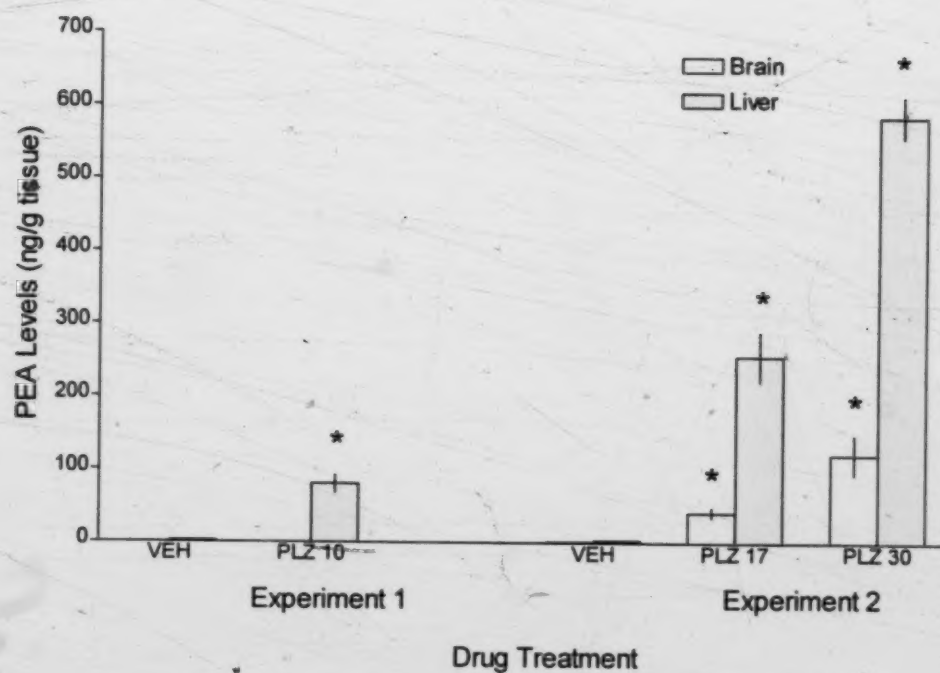


Figure 12. PEA levels in brain and liver of rats following chronic administration of VEH or PLZ (10, 17 and 30 mg kg⁻¹ p.o.). PEA levels were 2.1 ± 0.13 and 1.6 ± 0.2 ng/g of tissue in VEH-treated rats from experiments 1 and 2, respectively. Data shown are means ± SEM (n=15). *Effects are significant, relative to VEH, p<0.05, ANOVA.

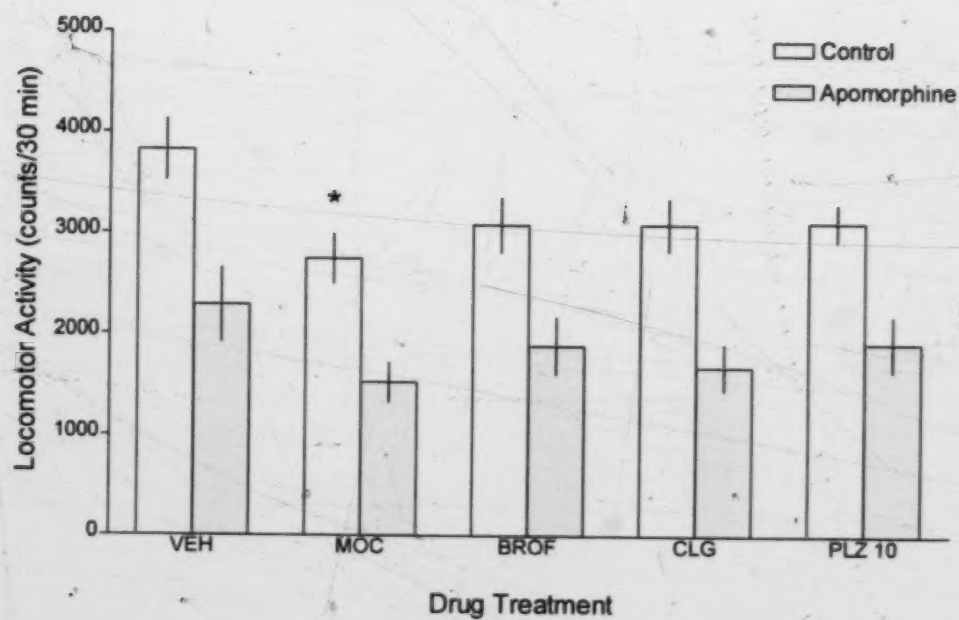


Figure 13. Effects of chronic (30 day) oral administration of VEH, MOC, BROF or PLZ on apomorphine-induced locomotor suppression in rats. Data shown are means $\pm$ SEM ($n=15$). *Effects are significant, relative to VEH, $p<0.05$, ANOVA.

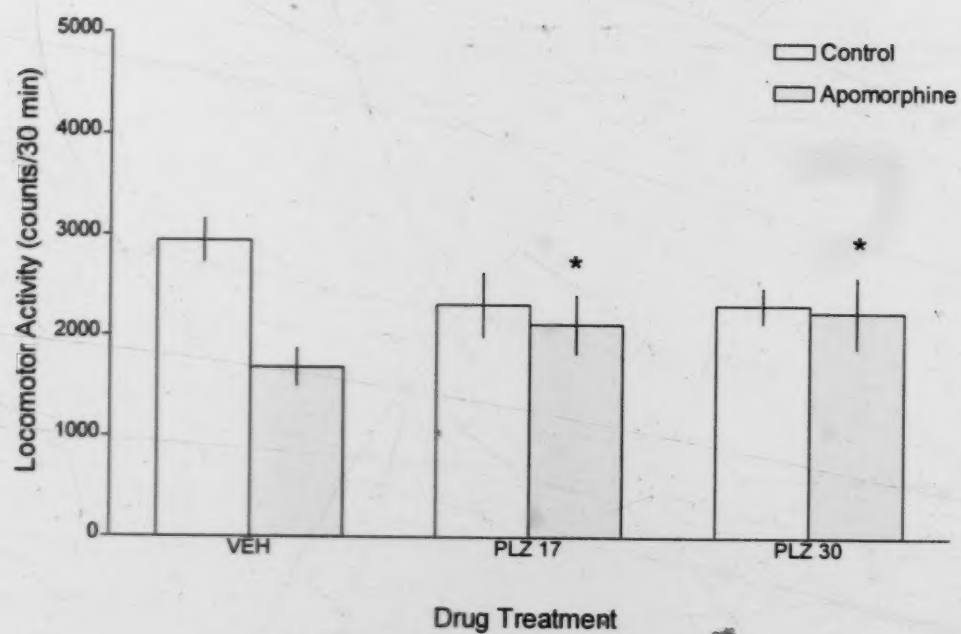


Figure 14. Effects of chronic (16 day) oral administration of VEH or PLZ (17 and 30 mg kg⁻¹) on apomorphine-induced locomotor suppression in rats. Both doses of PLZ attenuated the locomotor-suppressant effect of apomorphine. Data shown are means $\pm$ SEM (n=15). *Effects are significant, relative to VEH, $p < 0.05$, ANOVA.

(1 mg kg⁻¹) or the lowest dose of PLZ (10 mg kg⁻¹) despite adequate MAO inhibition (following administration of either inhibitor) and a significant reduction in ³H-tryptamine binding and in ³H-ketanserin binding to 5-HT_{2A} receptors following the administration of PLZ (Urichuk *et al.*, 1994). These results contrast with the results of previous studies (Allison *et al.*, 1993, 1995) in which CLG and PLZ, when administered chronically by the s.c. route, resulted in an attenuation of the locomotor-suppressant effects of apomorphine (PLZ) and quinpirole (PLZ and CLG) despite levels of MAO inhibition comparable to those obtained in the present study following oral administration. Therefore, although the previous study suggested that selective inhibition of MAO-A by CLG may be a sufficient condition for mediating the effects of MAO inhibitors on DA autoreceptor-mediated responses, the present findings indicate that MAO inhibition alone is not the sole factor responsible for mediating these effects. Following oral administration of PLZ, it may be that a change in PLZ or metabolite levels resulting from first pass metabolism, which is avoided with subcutaneous administration, may have altered the effects of PLZ which are mediated *via* a mechanism other than MAO inhibition. This is supported by the demonstrated equipotent ability of PLZ to inhibit MAO regardless of the route of administration whereas the behavioural effects are quite different.

Since treatment with MAO inhibitors has been shown to increase levels of trace amines (Boulton *et al.*, 1973; Axelrod and Saavedra, 1975; Boulton, 1976; Philips and Boulton, 1979; McKim *et al.*, 1980; Philips *et al.*, 1980), it may be that PLZ's clinical effects are partially mediated by the trace amines. Furthermore, it has

been shown that trace amines can alter uptake and/or release of catecholamines and 5-HT (Baker *et al.*, 1977, Raiteri *et al.*, 1977). PEA, an endogenous trace amine, is also a metabolite of PLZ (Baker *et al.*, 1982; Dyck *et al.*, 1985) which may be implicated in the etiology of depression (Dewhurst, 1968; Boulton *et al.*, 1974; Boulton and Milward, 1971; Sabelli *et al.*, 1974; Sandler *et al.*, 1979). The ability of PEA to increase the release of and inhibit the uptake of DA and NA (Raiteri *et al.*, 1977), respectively, also suggests a potential role for PEA in mediating the clinical effects of PLZ (Baker *et al.*, 1976, 1987).

In the second experiment of the present study, the effects of two higher doses of PLZ on apomorphine-induced locomotor suppression and PEA concentration in brain and liver were observed in order to explore the possibility that brain PEA levels may be important in mediating the effects of PLZ. Brain PEA levels have been found to be increased by CLG at 25 mg kg⁻¹ i.p. (Philips and Boulton, 1979). In the present study, however, the effects of a higher dose of CLG on apomorphine-induced locomotor suppression were not studied since it has been shown that at doses higher than 1 mg kg⁻¹ p.o. CLG loses its selectivity for inhibition of MAO type-A (Waldmeier *et al.*, 1981).

The inability of the 10 mg kg⁻¹ p.o. dose of PLZ to attenuate apomorphine-induced locomotor suppression despite brain and liver levels of MAO inhibition comparable to those obtained following subcutaneous administration of the same dose suggests that MAO inhibition is not a sufficient condition for mediating the effects of PLZ on DA autoreceptor-mediated responses. This dose of PLZ, however,

resulted in liver PEA levels eight times higher than those measured in previous studies (McManus *et al.*, 1991; Paetsch *et al.*, 1993) in which PLZ was administered at the same dose subcutaneously and attenuated the locomotor-suppressant effects of apomorphine, quinpirole and salbutamol (McManus *et al.*, 1991; McManus and Greenshaw, 1991; Allison *et al.*, 1993, 1995; Paetsch *et al.*, 1993). This may be due to the fact that PLZ is metabolized more to PEA in liver following oral versus subcutaneous administration. Baker *et al.* (1982) state that since PEA crosses the blood-brain barrier, it is possible that the conversion of PLZ to PEA occurs in the liver and that the PEA formed then enters the brain. This suggests that the brain concentration of PEA in the rats treated with a 10 mg kg⁻¹ p.o. dose of PLZ would be greater than that measured in the rats treated with 10 mg kg⁻¹ s.c. PLZ.

Although the brain and liver PEA levels were significantly different following the 17 and 30 mg kg⁻¹ doses of PLZ, there was no difference in the extent to which these two doses attenuated the locomotor-suppressant effect of apomorphine. Therefore, this lack of a dose-response relationship suggests that an increased PEA concentration is not a sufficient condition for mediating the effects of PLZ.

In a study by McManus *et al.* (1991), 5 and 10 mg kg⁻¹ s.c. doses of PLZ both inhibited MAO activity to approximately the same amount. The higher dose, however, resulted in a significant increase in brain and liver PEA levels and attenuated the motor-suppressant effect of salbutamol, a β -adrenoceptor agonist, while the lower dose had no effect. Since MAO inhibition was similar in both the low-dose and high-dose animals but the PEA levels and behavioural response to

salbutamol differed, this suggested that PEA may be important in mediating the effects of PLZ. Paetsch *et al.* (1993) replicated these results and also demonstrated that animals treated chronically (21-22 days) with a combination treatment of (-)-deprenyl (1 mg kg⁻¹ s.c.) and PEA (10 mg kg⁻¹ s.c.) exhibited an attenuation of the locomotor-suppressant effect induced by salbutamol. Animals treated chronically with either deprenyl or PEA alone did not exhibit this attenuation of salbutamol-induced locomotor suppression. However, despite the fact that PEA levels were much greater (~900% of control) in the animals which received the combined deprenyl/PEA treatment compared with those receiving only PLZ (~300% of control), the animals receiving the combination treatment did not show any greater attenuation of the response to salbutamol than that seen following chronic treatment with PLZ alone. These findings, in addition to those of the present study, indicate a lack of a dose-response relationship and suggest that factors other than PEA may play a role in mediating the effects of PLZ on apomorphine-induced locomotor suppression.

It could be argued that the discrepancy between the present results and those of Paetsch *et al.* (1993) and McManus *et al.* (1991) may be due to the possibility that PEA does not alter β -adrenoceptor-mediated effects in the same way that it alters DA autoreceptor-mediated effects. This seems unlikely, however, since this dose administered subcutaneously attenuated the locomotor-suppressant effect induced by apomorphine, quinpirole and salbutamol.

Both acute and chronic treatment of rats with PLZ have been reported to increase brain GABA levels (Baker *et al.*, 1982, 1991; McKenna *et al.* 1991;

Paslawski *et al.*, 1995, 1996; Todd and Baker, 1995). This effect, however, can be reversed by pretreatment with the MAO inhibitor, tranylcypromine (Todd and Baker, 1993). Pretreatment of PLZ with (-)-deprenyl, a selective MAO-B inhibitor, has been reported to reverse the PLZ-induced increase in brain GABA levels to a greater extent than that which is observed following pretreatment with CLG (Todd and Baker, 1995). These studies suggest that a metabolite of PLZ, produced by the action of MAO on PLZ, is responsible for increasing GABA levels and that MAO-B plays a more important role than MAO-A in the formation of this metabolite. In view of the first-pass metabolic effects encountered with oral administration, it was also of interest to measure GABA levels following oral administration.

In the present study, brain GABA levels were significantly different following the 17 and 30 mg kg⁻¹ doses of PLZ, yet there was no difference in the extent to which these two doses of PLZ attenuated the locomotor-suppressant effect of apomorphine. This lack of a dose-response relationship suggests that GABA is also not a sufficient condition for mediating the behavioural effects of PLZ.

Although the levels of DA, NA and 5-HT were significantly higher following PLZ (17 and 30 mg kg⁻¹) *versus* those obtained following VEH administration, there was no difference between these levels obtained following the 17 and 30 mg kg⁻¹ doses of PLZ. There was also no difference with respect to the extent to which these two doses attenuated apomorphine-induced locomotor suppression. No other studies to date have compared the effects of orally administered PLZ at comparable doses, in rats, on brain biogenic amine levels. Furthermore, no other studies have investigated the relationship between the effects of PLZ at these

doses on biogenic amine levels and locomotor activity. It is, therefore, difficult to draw any conclusions concerning the potential role of these biogenic amines in mediating the behavioural effects of PLZ.

Although the metabolism of PLZ appears to be quite extensive, it is still poorly understood (Baker *et al.*, 1998). Furthermore, no studies have compared the effects of s.c. and p.o. administration of PLZ on the types and levels of metabolites obtained; the present results indicate that such investigations in future would be warranted. The present results and the observations described above indicate that although MAO inhibition and increased PEA, GABA and biogenic amine levels may play a role in mediating the effects of PLZ on apomorphine-induced locomotor suppression, it appears that other factors may contribute to the mechanism of action of PLZ. Thus, further studies are required to determine the mechanisms involved in mediating the effects of chronic PLZ administration on DA autoreceptor-mediated functional responses.

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CHAPTER 7

Chronic Treatment of Rats with Fluoxetine or Trimipramine Does Not Attenuate Quinpirole-Induced Locomotor-Suppression

7.1. Introduction

Fluoxetine (FLU) is a SSRI which has been shown to have good efficacy in treating depression, eating disorders such as obesity and bulimia (Freeman and Hampson, 1987; Marcus *et al.*, 1990) and obsessive-compulsive disorder (Jenike *et al.*, 1989). It is thought that the antidepressant effect of FLU is a result of its ability to selectively enhance serotonergic transmission (Benfield *et al.*, 1986; Preskorn, 1996). It has been found to have very little affinity for α - or β -adrenergic, cholinergic, histaminic H_1 , opiate or DA receptors (Stark *et al.*, 1985; Wong *et al.*, 1985). Interestingly, FLU has a lower affinity for 5-HT₁ or 5-HT₂ receptors than several other antidepressants (Stark *et al.*, 1985).

Several lines of evidence suggest that the 5-HT system may somehow modulate the DA system. Neuroanatomical studies have shown that there are 5-HT neurons which originate in the median and dorsal raphe nuclei and terminate in the substantia nigra and VTA (Azmitia and Segal, 1978; Steinbush, 1984; Jacobs and Azmitia, 1992). Despite the neuroanatomical evidence which suggests a possible interaction between 5-HT and DA, there are conflicting reports with respect to whether the effect of 5-HT on DA is stimulatory or inhibitory. These differential effects of 5-HT on DA neuronal activity may stem from the stimulation of different 5-HT receptor subtypes (Blandina *et al.*, 1989; Kalivas, 1993; Prisco *et al.*, 1994).

The data from some studies suggest a facilitatory effect of 5-HT on dopaminergic neurons. A study by Van Bockstaele *et al.* (1994) examined the synaptic structure of 5-HT terminals on neurons in the VTA which project to the

nucleus accumbens and determined that the predominance of asymmetric junctions formed by these 5-HT terminals onto DA neurons in the VTA suggests a primarily excitatory influence of 5-HT on the dopaminergic neurons. Guan and McBride (1989) have demonstrated that the infusion of 5-HT into the VTA of rats enhances DA release in the nucleus accumbens. This facilitatory release of DA by 5-HT has also been observed in rat striatum (Benloucif and Galloway, 1991). In addition, electrical self-stimulation of the medial forebrain bundle is enhanced by the administration of 5-HT into the ventral midbrain (Fiorino *et al.*, 1993).

Conversely, the results of other studies suggest that 5-HT may exert a tonic inhibitory influence on DA transmission. Lesions in the median and dorsal raphé nuclei result in an increase in striatal levels of DOPAC and HVA while DA is not affected (Juorio and Greenshaw, 1986). The anti-immobility effects of FLU in the forced swimming test are decreased by the administration of ($\pm$)-sulpiride, a D_2 DA receptor antagonist (Cesana *et al.*, 1993). 8-OH-DPAT, a 5-HT_{1A} receptor agonist which inhibits 5-HT neuronal firing in both dorsal and median raphé nuclei, has been shown to potentiate the basal firing rate of dopaminergic neurons in the VTA (Prisco *et al.*, 1994).

Prisco *et al.* (1994) have also demonstrated that the activity of dopaminergic neurons in the VTA is decreased by the 5-HT_{2C/2B} receptor agonists mCPP and TFMPP. These same effects were also demonstrated in the substantia nigra (Kelland *et al.*, 1990). These findings, which seem to suggest that the 5-HT system may exert a tonic inhibitory effect on the dopaminergic system, are further

supported by Parkinson-like extrapyramidal side-effects which can occur in patients being treated for depression with FLU (Bouchard *et al.*, 1989). Furthermore, the reported ability of FLU to potentiate the discriminability of cocaine suggests that it may be mediating some of its effects *via* the DA system (Simon and Appel, 1997). These studies suggest that, despite its lack of affinity for other receptors (Wong *et al.*, 1983; Stark *et al.*, 1985), it is possible that FLU may mediate some of its effects by the DA system.

In previous studies (Allison *et al.*, 1993,1995), chronic administration of CMI, a potent 5-HT uptake inhibitor, failed to attenuate the locomotor-suppressant effects of low doses of both apomorphine and quinpirole (which are believed to be selective for presynaptic DA autoreceptors). These results suggested that selective inhibition of 5-HT uptake may not lead to a down-regulation of DA presynaptic autoreceptor function. The N-demethylated metabolite of CMI, DMCMI, is more potent at inhibiting the uptake of NA than 5-HT (IC_{50} = .46 vs. 41 nM, respectively)(Hyttel, 1982). Consequently, CMI cannot be considered as a SSRI, even though in the rat, DMCMI is found in very low amounts (Allison *et al.*, 1993,1995; Fung *et al.*, 1994). In the present study, the effect of chronic treatment with FLU, a SSRI, on the behavioural response to low-dose quinpirole was assessed in order to determine the effects of chronic selective inhibition of 5-HT uptake on presynaptic DA autoreceptors. The effect of chronic treatment with the TCA TMP was also studied since its mechanism of action is currently unknown (Hauser *et al.*, 1985; Yu and Boulton, 1990; Gross *et al.*, 1991).

7.2. Materials and Methods

7.2.1. Animals and Surgery

Male Sprague-Dawley rats weighing between 200 and 250 g at the time of surgery were used. Animals were randomly allocated to one of three drug treatment groups ($n=13-15$) that received either FLU HCl, TMP maleate, or VEH (dimethylsulfoxide, DMSO: 25%v/v in distilled water). Antidepressant drug doses were 10 mg kg^{-1} based on free base weights. Animals were implanted with 2ML4 Alzet osmotic minipumps under aseptic conditions as described in the General Methods section 2.2.13. After 28 days of continuous drug infusion rats were killed by guillotine decapitation, the brains rapidly removed, frozen over dry ice and stored at -80°C until used for analysis of drug and biogenic amine levels. Drug level measurements were taken to ensure the efficacy of drug administration and were compared with levels previously obtained in this laboratory.

7.2.2. Drug Levels

Brain tissue concentrations of FLU, TMP, and their demethylated metabolites norfluoxetine (NFLU) and N-desmethyldesipramine (NTMP), respectively, were determined by gas chromatography with NPD using a modification of the procedure of Drebit *et al.* (1988) as described in the General Methods section 2.2.18.

7.2.3. Levels of Biogenic Amines and Acid Metabolites

Brain levels of the biogenic amines DA and 5-HT and their metabolites,

HVA, DOPAC and 5-HIAA as well as of NA and TRP were determined using HPLC with electrochemical detection using the methodology and apparatus as described in the General Methods section 2.2.21.

7.2.4. Behavioural Testing

On days 21 and 22 of chronic drug or VEH administration, the effects of either (-)-quinpirole HCl (0.05 mg kg^{-1}) or distilled water on spontaneous locomotor activity were measured. The dose of quinpirole is expressed as the free base. Either quinpirole or the distilled water VEH was injected subcutaneously in a volume of 1 ml kg^{-1} 5 min prior to each behavioural test. Spontaneous locomotor activity was measured by placing animals individually in a computer-controlled infrared activity system for 30 min as described in the General Methods section 2.2.14.

7.2.5. Statistics

Effects of drug treatment on locomotor activity, drug and biogenic amine/metabolite levels were analysed using analysis of variance (ANOVA). In the case of a significant F test, a Newman-Keuls multiple comparison test was used to determine significant differences between means ($\alpha=0.05$) (Winer, 1971).

7.3. Results

7.3.1. Drug Levels

Concentrations of FLU and NFLU in the brain tissue of rats treated with FLU

were $2.06 \pm .14$ (6659 ± 452 nM) and 34.53 ± 3.60 (117320 ± 12230 nM) $\mu\text{g/g}$ of tissue, respectively (Table 5). The concentration of TMP was 320 ± 30 ng/g of brain tissue (1086 ± 102 nM) while NTMP, if present, was below the detectable limits of our assay, i.e. 75 ng/g tissue.

7.3.2. Levels of Biogenic Amines and Acid Metabolites

In brain tissue of FLU-treated rats, 5-HIAA levels were significantly lower than those of VEH-treated rats (Table 6) at a value of 157.8 vs. 244.38 ng/g of tissue, respectively. Levels of DA, 5-HT, HVA, DOPAC, NA and TRP were no different from controls. In TMP-treated rats, brain levels of DA and 5-HT were lower than those which were measured in VEH-treated rats (Table 6). DOPAC levels were higher relative to control levels. HVA, 5-HIAA, NA and TRP levels were unaffected.

7.3.3. Locomotor Activity

For chronic VEH-treated rats, locomotor counts were 2869 ± 228 counts/30 min following the acute VEH injection. The corresponding locomotor counts for rats treated with FLU and TMP were 3042 ± 242 and 2767 ± 194 counts/30 min, respectively. Statistical analysis indicated that there was no significant effect of the chronic antidepressant treatment alone on locomotor activity [$F(2,41) = 0.514$, $p > 0.05$]. There was a significant effect of quinpirole on spontaneous locomotor activity [$F(1,41) = 59.2$, $p < 0.05$] as illustrated in Figure 15.

In chronic VEH-treated rats, locomotor activity was reduced by quinpirole to $54 \pm 19\%$ of the control level. There was, however, no significant interaction

	FLU (n=15)	TMP (n=15)
FLU	2.06 ± .14	-
NFLU	34.53 ± 3.60	-
TMP	-	.321 ± .03

Table 5. Drug levels ($\mu\text{g/g} \pm \text{SEM}$, $n=15$) in whole brain following continuous infusion of FLU (10mg kg^{-1}) and TMP (10mg kg^{-1}) via osmotic minipumps for 28 days.

	VEH (n=13)	FLU (n=15)	TMP (n=15)
DA	630.54 ± 35.29	684.58 ± 18.01	504.64 ± 17.25*
DOPAC	91.92 ± 15.03	84.54 ± 5.39	142.50 ± 15.69*
HVA	59.26 ± 3.21	59.94 ± 2.62	68.06 ± 3.77
5-HT	244.80 ± 20.85	254.34 ± 10.33	158.11 ± 13.66*
5-HIAA	244.38 ± 7.99	157.82 ± 6.71*	279.58 ± 32.06
NA	393.74 ± 7.02	370.30 ± 7.66	357.36 ± 16.25
TRP	3.56 ± 0.48	4.07 ± 0.18	4.24 ± 0.73

Table 6. Levels of biogenic amines and their metabolites (ng/g ± SEM) and TRP (µg/g ± SEM) in whole brain following continuous infusion of FLU (10mg kg⁻¹) and TMP (10mg kg⁻¹) via osmotic minipumps for 28 days.

*Effects were significant at p<0.05, relative to VEH.

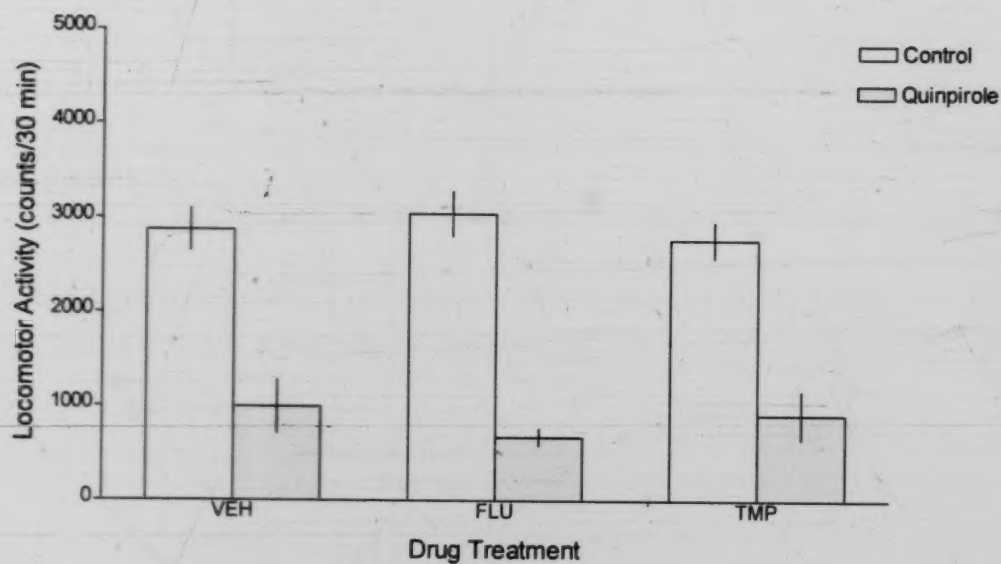


Figure 15. Effect of quinpirole (0.05 mg kg^{-1} , s.c., 5 min) on spontaneous locomotor activity of rats following chronic VEH or antidepressant treatment (s.c. via osmotic minipump). FLU and TMP did not attenuate quinpirole-induced locomotor suppression. Data shown are means $\pm$ SEM ($n=15$).

between the chronic antidepressant treatment and the acute treatment with quinpirole [$F(2,41) = 0.39$, $p > 0.05$].

7.4. Discussion

In the present study, chronic administration of either FLU or TMP had no effect on locomotor suppression induced by a low dose of quinpirole. The absence of effect by FLU supports our proposal based on previous findings with chronic CMI treatment (Allison *et al.*, 1993, 1995) that inhibitors of 5-HT uptake do not mediate their effects by decreasing presynaptic DA autoreceptor function. These results are also consistent with those of Collu *et al.* (1997) who reported no effect of chronic FLU administration on quinpirole-induced locomotor suppression.

Despite FLU's lack of affinity for DA as well as other receptors (Stark *et al.*, 1985; Wong *et al.*, 1985), several lines of evidence suggest that 5-HT may modulate dopaminergic transmission and thus may mediate its effects *via* the DA system. The antagonistic effect on FLU's anti-immobility response by ($\pm$)-sulpiride, a D_2 DA receptor antagonist, in the forced swimming test supports a possible role for DA in mediating FLU's effects (Cesana *et al.*, 1993). Furthermore, mCPP, a 5-HT_{2C/2B} receptor agonist, decreases the firing rate of DA in neurons in the VTA of control rats. Following chronic administration of FLU, however, this effect of mCPP is absent, suggesting a downregulation of 5-HT_{2C/2B} receptors. Thus, these data suggest that FLU may mediate its effects on the DA system via the 5-HT_{2C/2B} receptors (Prisco and Esposito, 1995).

Prisco and Esposito (1995) have demonstrated that acute administration of FLU dose-dependently decreased the firing rate of DA neurons in the VTA but was without effect in the substantia nigra. When an acute FLU injection was administered 72 h after the last of the chronically administered FLU injections, there was no inhibition of the firing rate of DA neurons as seen following only an acute administration of FLU. Thus, mCPP inhibited DA neuron firing in control rats but had no effect on DA function in rats treated chronically with FLU. These data seem to suggest that FLU may modulate DA firing in the VTA by the 5-HT_{2C/2B} receptor.

The presentation of extrapyramidal side effects in some patients taking FLU or other SSRIs (Bouchard *et al.*, 1989) also suggests a possible link between 5-HT and DA systems. Baldessarini and Marsh (1990) have demonstrated that chronic treatment with FLU resulted in a decreased synthesis of DA in the rat striatum, nucleus accumbens and frontal cortex, suggesting that 5-HT may exert an inhibitory effect on DA transmission. In a later study, however, Baldessarini *et al.* (1992) were unable to replicate these results although they did observe a significant decrease in 5-HT synthesis as indicated by a reduction in the accumulation of the 5-HT precursor 5-hydroxytryptophan (5-HTP) in rat striatum, hippocampus and frontal cortex but not in the nucleus accumbens.

DA levels have been shown to increase in the prefrontal cortex but not the nucleus accumbens following acute treatment with FLU (Tanda *et al.*, 1994). Perry and Fuller (1992), however, have found that although the 5-HT metabolite 5-HIAA was significantly decreased, there was no change in levels of DA or those of its

metabolites, DOPAC and HVA in rat striatum.

In the present study, the levels of FLU and NFLU measured in rat brain are consistent with those found previously in our laboratory. Neither DA nor 5-HT levels in the present study were significantly different from those found in control rats, although 5-HIAA levels were reduced. The decrease in 5-HIAA levels is consistent with what other studies have demonstrated following chronic FLU administration (Hwang *et al.*, 1980; Hrdina *et al.*, 1987; Caccia *et al.*, 1992; Trouvin *et al.*, 1993; Kreiss and Lucki, 1995). Beasley *et al.* (1992) suggest that the reduced concentration of 5-HIAA which results subsequent to chronic treatment with FLU in the absence of any changes in 5-HT concentration may be indicative of the inhibition of 5-HT uptake by FLU and, consequently, a decreased access of 5-HT to the presynaptic terminal and subsequent degradation by MAO.

There is some discrepancy among studies reported in the literature as to whether 5-HT levels are increased, decreased, or unchanged (Hwang *et al.*, 1980; Savage *et al.*, 1980; Hrdina *et al.*, 1987; Rutter *et al.*, 1994) following chronic FLU administration. The absence of a change in 5-HT levels following chronic FLU administration in the present study is consistent with the findings of Hwang *et al.* (1980) and Savage *et al.* (1980).

In any case, the lack of attenuation of the quinpirole-induced locomotor-suppressant effect following chronic treatment with FLU is consistent with previous studies (Allison *et al.*, 1993; 1995) where chronic administration of CMI, a potent 5-HT uptake inhibitor, had no effect on apomorphine- or quinpirole-induced locomotor

suppression. Mousseau and Greenshaw (1989) have demonstrated that chronic (28 days) treatment with CMI results in decreased 5-HIAA levels in frontal cortex, hippocampus, caudate nucleus and hypothalamus without a change in 5-HT levels consistent with the ability of CMI to inhibit 5-HT uptake. These previous results and those of the present study suggest strongly that 5-HT uptake-inhibiting antidepressants do not mediate their effects by decreasing DA presynaptic autoreceptor function.

Chronic administration of TMP also did not attenuate the locomotor-suppressant response to low-dose quinpirole. Although the chemical structure of TMP is very similar to that of imipramine, the mechanism of action does not appear to be imipramine-like and is currently unknown. In spite of this, the efficacy of TMP as a treatment for depression has been found to be equal to that of other antidepressants (Abernathy *et al.*, 1984; Assalian *et al.*, 1985; Lapierre *et al.*, 1989). It has also been shown to have good efficacy as an anxiolytic (Assalian *et al.*, 1985; Pecknold *et al.*, 1985, 1989) and an antipsychotic (Eikmeier *et al.*, 1985; Gross *et al.*, 1991).

Unlike most TCAs, TMP does not decrease β -adrenoceptor density (Hauser *et al.*, 1985). TMP is only a very weak inhibitor of DA, NA and 5-HT uptake (Hyttel, 1982; Richelson and Pfenning, 1984) and does not appear to alter 5-HT or NA presynaptic autoreceptor function (Gross *et al.*, 1991). Although it has, like imipramine (Roth and Gillis, 1974), been reported to act as a weak reversible inhibitor of MAO-B, it has not been found to have any effect on the activity of other

metabolic enzymes such as phenylalanine hydroxylase, dopamine- β -hydroxylase, tyrosine hydroxylase, COMT, or L-AAD (Yu and Boulton, 1990).

TMP's affinity for D₂ DA receptors (Gross *et al.*, 1991) and its ability, following acute treatment, to increase DOPA accumulation in the presence of a L-AAD inhibitor (implying an increase in DA synthesis) (Carlsson and Lindqvist, 1978), suggests that TMP may mediate its effects via the DA system. Acute injections of TMP at 30 mg kg⁻¹ have been shown to increase DA synthesis without any effect on 5-HT synthesis (Carlsson and Lindqvist, 1978). Therefore, it may be that TMP mediates its effects, at least in part, via the DA system.

In a study by Delini-Stula *et al.* (1988) both TMP and levoprotiline reduced the immobility of rats in a forced swimming test. The D₂ DA receptor antagonists, haloperidol and ($\pm$)-sulpiride, but not the α_1 -adrenergic antagonist, prazosin, attenuated this effect of levoprotiline, suggesting that the anti-immobility effects of this antidepressant may be mediated through the DA system. The effects of these antagonists on TMP-induced reduction of immobility were not investigated.

In the present study, a decrease in the levels of DA and 5-HT, and an increase in DOPAC with no change in HVA, 5-HIAA, NA or TRP were found in TMP-treated rats. The absence of any change in NA or 5-HIAA levels in the present study following chronic TMP is consistent with the lack of effect on NA-stimulated adenylate cyclase, β -adrenoceptor density (Hauser *et al.*, 1985), 5-HT uptake or presynaptic 5-HT and NA autoreceptor function (Gross *et al.*, 1991). Juorio *et al.* (1990) have demonstrated that following chronic administration of TMP, levels of DA

were increased in the striatum, nucleus accumbens and olfactory tubercles but were unchanged in the frontal cortex. In addition, they found a decrease in the number of D₂ DA receptors in the striatum but not the nucleus accumbens or frontal cortex. Juorio *et al.* (1990) also found an increase in 5-HT and 5-HIAA in the frontal cortex, olfactory tubercles, hippocampus and hypothalamus but not in the striatum. There was no change in the levels of HVA, DOPAC or TRP in these brain regions.

It is difficult to understand the inconsistencies between the results of the present study and those of Juorio *et al.* (1990). In both studies, TMP was administered by osmotic minipump. In the present study, however, TMP was administered at 10 mg kg⁻¹ for 28 days, whereas, Juorio *et al.* (1990) administered TMP at 5 mg kg⁻¹ for 14 days. Furthermore, brain levels of TMP were measured in the present study while this was not carried out by Juorio *et al.* (1990). Consequently, it is difficult to directly compare the two studies with respect to the effects of specific TMP brain concentrations on the levels of biogenic amines and their metabolites. In the study of Juorio *et al.* (1990) biogenic amine levels were measured in specific brain regions whereas in the present study, biogenic amine levels were measured in whole brain. If chronic administration of TMP results only in regionally specific changes in 5-HT levels, then it is possible that such effects may have been masked by whole brain measurements.

Despite evidence suggesting that FLU and TMP may, in some way, mediate their effects *via* the DA system, the results obtained from the present study indicate that the SSRI, FLU, and TMP, a TCA whose mechanism of action is unknown, do

not mediate their effects by downregulating presynaptic DA autoreceptor function.

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CHAPTER 8

Effects Of Apomorphine On K^+ -Stimulated [^3H]DA Release In Rat Nucleus Accumbens

8.1. Introduction

In previous studies (Allison *et al.*, 1993,1995) the locomotor-suppressant effects of low doses of the DA receptor agonists apomorphine and quinpirole were attenuated by the TCA DMI and the MAOIs PLZ and CLG. In addition, PLZ and CLG, but not DMI, attenuated quinpirole-induced yawning which is thought to be mediated by D₃ DA receptors (Damsma *et al.*, 1993). Although both apomorphine and quinpirole are D₂-like DA receptor agonists, quinpirole has a higher affinity for D₃ versus D₂ DA receptors, while apomorphine has a similar affinity for D₂ and D₃ DA receptors (Sokoloff *et al.*, 1990).

Chronic administration, in rats, of TCAs such as DMI has been shown to decrease the density of D₁-like DA receptors whereas MAOIs such as PLZ may result in a downregulation of both D₁-like and D₂-like DA receptors (Paetsch and Greenshaw, 1992). The discrepancy between the ability of DMI to attenuate quinpirole-induced locomotor suppression but not yawning suggests that these two behaviours may be mediated by different mechanisms and may also reflect a possible difference between the mechanism of action of TCAs and MAOIs. Thus, it is possible that MAOIs such as PLZ and CLG may be mediating their effects through D₃ DA receptors.

In order to elucidate the differences between the possible mechanisms of action of the TCAs and the MAOIs as well as to further characterize the DA receptor agonists as to their DA receptor subtype selectivity, my objective was to compare the effects of apomorphine, quinpirole and 7-OHDPAT on K⁺-stimulated DA release

in the nucleus accumbens, which contains both D₂ and D₃ DA receptors versus the striatum, which has D₂ but very few if any D₃ DA receptors (Sokoloff *et al.*, 1990; 1992b). 7-OHDPAT, a DA receptor agonist which has been reported to be 7-100 times more selective for D₃ than for D₂ DA receptors (Levesque *et al.*, 1992; Sautel *et al.*, 1995), is believed to be more potent at D₃ DA receptors than quinpirole (Levesque *et al.*, 1992; Damsma *et al.*, 1993).

It was proposed that the extent to which each of these DA receptor agonists altered the evoked DA release in the nucleus accumbens versus the striatum would reflect the different affinities of these DA agonists for D₂ versus D₃ DA receptors (Farnebo and Hamberger, 1973; Yamada *et al.*, 1994). Furthermore, this information would be important in further determining the DA receptor subtype through which some antidepressants may mediate their effects. In the present experiment, the effects of a range of concentrations of apomorphine on K⁺-stimulated [³H]DA release in the rat nucleus accumbens were examined.

8.2. Materials and Methods

The superfusion procedure followed in this experiment was taken from the study of Baker *et al.* (1980) and is a modification of the procedure developed by Raiteri *et al.* (1974, 1975). Drug-naive rats were killed by decapitation and their brains rapidly removed. The nucleus accumbens was immediately dissected on an ice-cooled petri-dish. The nucleus accumbens (pooled from both hemispheres) from 2 rats was required for 1 superfusion run. The tissue was chopped on a

Mcllwain tissue chopper, yielding prisms of approximately 0.1 x 0.1 x 2 mm. The tissue prisms were then suspended in 5 volumes of an ice-cold control (5 mM K⁺) incubation medium of the following composition: 123 mM NaCl, 5 mM KCl, 2.7 mM CaCl₂, 1.2 mM MgSO₄, 20 mM Tris-HCl buffer, pH 7.4, 10 mM glucose, 12.5 μM nialamide (inhibitor of MAO) and 1 mM ascorbic acid.

In each superfusion run there were four different incubation medium conditions: 5 mM K⁺ (control), 15 mM K⁺, apomorphine made up in 5 mM K⁺ (apo/5 mM K⁺), and apomorphine made up in 15 mM K⁺ (apo/15 mM K⁺). Apomorphine, was made up in the appropriate incubation medium to yield the following concentrations: 0.1, 0.3, 1, 3, 10, and 30 μM. 15 mM K⁺ incubation medium was of the same composition as the 5 mM K⁺ incubation medium except that the concentration of NaCl was reduced to 113 mM.

The tissue was then further diluted with the incubation medium to yield a 5 mg/mL suspension which was then pre-incubated at 37°C in a shaking waterbath for 10 minutes. Radiolabelled ([³H]) DA (sp. act. 17.6 Ci/mmol) was then added (final concentration: 0.02 μM) and incubation was continued for a further 10 minutes. The tissue was subsequently separated from the incubation medium by rapid filtration through the six parallel superfusion chambers of a Brandel Superfusion 6 apparatus, each of which contained a Millipore filter at the base of the chamber. The superfusion chambers were thermostatically maintained at 37°C.

The tissue was washed by connecting the stem of the bottom plug of the chamber to a vacuum line and pouring 1 ml (twice) of 5 mM K⁺ incubation medium into the chamber (from the top) over the tissue. The intake tubing for each chamber

was then attached to a peristaltic pump, allowing the incubation medium (or apomorphine) to be drawn over the tissue at a rate of 1 ml/minute. There was a 6 min washout after which the intake was switched from the reservoir containing 5 mM K⁺ incubation medium to the sample tubes containing the drug (apo/5 mM K⁺ and apo/15 mM K⁺) and incubation medium (5 and 15 mM K⁺) of interest.

With a dead volume of 2 ml, the drug in question reached the tissue in time for release effects to be evident beginning at the second fraction. After collection of fraction number six, the intake was switched back to reservoir containing the control incubation medium. Fractions (0.5 min) were collected, yielding 0.5 ml of superfusate per liquid scintillation vial. At the completion of a superfusion run the filters from each superfusion chamber were removed and placed in their respective vials. Beckman® scintillation fluid (5 ml) was added to each filter and to each superfusate-containing vial. The amount of radioactivity in each fraction as well as in each filter was then counted using liquid scintillation spectrometry and expressed as % fractional release.

Calculation of % Fractional Release:

% fractional release refers to the amount of radioactivity in each superfusate fraction expressed as a percent of the total radioactivity which existed in the tissue at the onset of that fraction collection. The amount of radioactivity in each fraction is determined by dividing the amount of radioactivity in a specific fraction by the summed amount of radioactivity measured in the filter and each fraction which was

collected subsequent to the fraction in question. Multiplying the resultant value by 100 will yield the % fractional release for each respective vial.

8.2.1. Statistics

The effect of a range of concentrations of apomorphine on K⁺-stimulated [³H]DA release was analysed using analysis of variance (ANOVA) followed by a Multiple F planned comparisons test to determine the critical difference between the means ($\alpha = 0.05$) (Kreis, 1989).

8.3. Results

Statistical analysis indicated that there was a significant effect of 15 mM K⁺ incubation medium on basal [³H]DA release from nucleus accumbens prisms relative to control (5 mM K⁺ incubation medium) [$F(1,130) = 267.72, p < 0.05$]. None of the concentrations of apomorphine except for the 30 μ M concentration [$F(6,130) = 4.48, p < 0.05$] exhibited any effect on basal [³H]DA release and in this case it was only over a few fractions. The 3 μ M concentration of apomorphine significantly inhibited 15 mM K⁺-stimulated [³H]DA release [$F(6,130) = 3.39, p < 0.05$] (Figure 16) but none of the other concentrations had any effect (data not shown).

8.4. Discussion

In the present study, there was evidence of a 15 mM K⁺-stimulated [³H]DA release from rat nucleus accumbens prisms. Of the range of concentrations (0.1 -

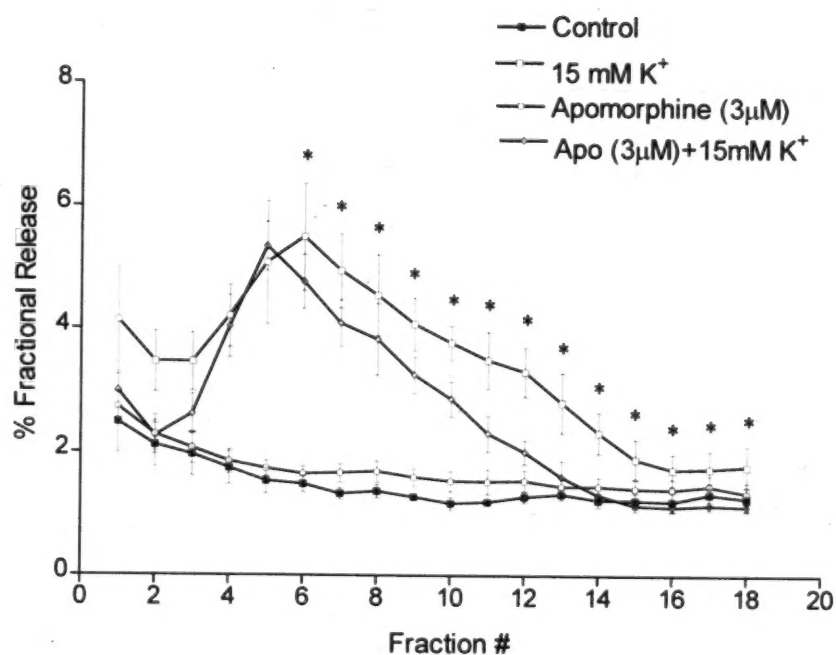


Figure 16. Effect of 3 μ M apomorphine on 15 mM K⁺-stimulated [³H]DA release from rat nucleus accumbens prisms. 3 μ M apomorphine reduced 15 mM K⁺-stimulated [³H]DA release at fractions 6-18. Data shown are means $\pm$ SEM of 4-8 experiments. *Effects were significant at $p < 0.05$, ANOVA.

30 μM) of apomorphine studied, however, only the 3 μM concentration reduced the release of [^3H]DA evoked by the 15 mM K^+ incubation medium.

Previous studies (Allison *et al.*, 1993, 1995) suggested that antidepressants such as DMI, PLZ and CLG may mediate their effects by the DA system but it was unclear as to which DA receptor subtype (D_2 versus D_3) they were acting on. To determine the selectivity of apomorphine and quinpirole for D_2 versus D_3 DA receptors, the extent to which these two DA receptor agonists alter evoked release were to be measured and compared in the nucleus accumbens, which contains both D_2 and D_3 DA receptors, versus the striatum, which contains D_2 but very few, if any, D_3 DA receptors (Sokoloff *et al.*, 1990). Results from the present study, however, suggested that K^+ stimulation may not be a reliable model with which to access the presynaptic autoreceptors in nucleus accumbens prisms.

Most investigations to date have studied the effects of stimulated DA release in brain slices rather than prisms. When comparing the results of different studies, there seems to be a greater number of inconsistencies with respect to the effect of apomorphine on stimulated [^3H]DA release when using K^+ versus electrical stimulation as a method of depolarizing nerve terminals. It may be that electrical stimulation is more comparable to the action potential which occurs under physiological conditions.

Farnebo and Hamberger (1971) observed that apomorphine at 0.1, 1 and 10 μM concentrations caused a slight decrease of electrically-induced [^3H]DA release from rat striatal slices but they did not discuss the statistical significance of these

results. Starke *et al.* (1978) observed that 0.01 - 10 μ M apomorphine inhibits electrically-stimulated [^{3} H]DA release from rabbit striatal slices. While 0.1 μ M apomorphine was found to inhibit electrically-stimulated [^{3} H]DA release from rabbit striatal slices in a study by Arbilla and Langer (1981), their previous study (Arbilla and Langer, 1978) demonstrated that 0.1 - 10 μ M apomorphine had no effect on 30 mM K $^+$ -stimulated [^{3} H]DA release from rat striatal slices. Raiteri *et al.* (1978) also found no effect of 1 μ M apomorphine on 30 mM K $^+$ -stimulated [^{3} H]DA release from rat striatal synaptosomes. On the other hand, Miller and Friedhoff (1979) found that of a range of concentrations (0.01 - 100 μ M) of apomorphine, only 0.1 μ M apomorphine reduced the 33 mM K $^+$ -stimulated [^{3} H] DA release from rat striatal slices.

In an attempt to determine if drug-induced effects on DA release are related to their affinity to different DA receptor subtypes, Yamada *et al.* (1994) observed that apomorphine, quinpirole and 7-OHDPAT differentially influenced electrically-induced DA release from rat nucleus accumbens and striatal slices. Apomorphine, which has equal affinity for D $_2$ and D $_3$ DA receptors (Sokoloff *et al.*, 1990; 1992a) reduced electrically-induced DA release from both the nucleus accumbens and striatum to the same extent. Quinpirole and 7-OHDPAT, which each have a greater affinity for D $_3$ DA receptors (Sokoloff *et al.*, 1990, 1992a; Levesque *et al.*, 1992; Damsma *et al.*, 1993) preferentially reduced the electrically-induced DA release in the nucleus accumbens versus the striatum.

In summary, the results of the abovementioned studies as well as those

presented in the present study suggest that using high K^+ as a method of depolarizing nerve terminals in prisms in order to assess the affinity of DA receptor agonists for presynaptic autoreceptors may not be as reliable as electrical stimulation as a model with which to access these receptors in the nerve terminals. Furthermore, it may be that the integrity of cellular components in presynaptic nerve terminals is greater in slices than in prisms to the extent that it alters the ability to access presynaptic autoreceptors.

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Chapter 9

General Discussion

9.1. GENERAL DISCUSSION

There is a general consensus which postulates that antidepressants may mediate their effect by enhancing the neurotransmission of biogenic amines. In addition to NA and 5-HT, the potential role of DA in the etiology and treatment of depression is becoming increasingly apparent. Several lines of evidence support the role of DA in the etiology and treatment of depression. The reported role of DA in mediating reward and the fact that anhedonia is a core symptom of depression suggests a possible relationship between DA and depression.

The locomotor-stimulant responses to DA receptor agonists are reported to be potentiated following chronic administration of antidepressant drugs (Maj *et al.*, 1987; Serra *et al.*, 1990; Spyraiki and Fibiger, 1981). These studies have suggested that the effects of chronically administered antidepressants may be mediated by a postsynaptic DA receptor supersensitivity. This theory, however, has been difficult to reconcile with the reported decrease in density of D₁ and D₂ DA receptors following chronic treatment with TCAs (D₁) (Klimek and Maj, 1990; Klimek and Nielsen, 1987; Paetsch and Greenshaw, 1992), and MAO inhibitors (D₂) (Paetsch and Greenshaw, 1992).

Chronic administration of antidepressants has been shown to attenuate the locomotor-suppressant effect of low doses of DA receptor agonists which are believed to be selective for presynaptic DA autoreceptors (Serra *et al.*, 1979; Maj *et al.*, 1989; Allison *et al.*, 1993, 1995). This suggests that the effects of chronically administered antidepressants may be mediated by a decrease in presynaptic DA

autoreceptor function.

In the experiments presented in this thesis, the possibility that different antidepressants may be mediating their effects by decreasing DA presynaptic autoreceptor function was investigated by observing the effects of chronic antidepressant treatment in rats on the locomotor suppression induced by low doses of the DA receptor agonists, apomorphine and quinpirole, which are believed to be selective for presynaptic DA autoreceptors.

In chapter 3, chronic administration of the TCA, DMI, and the MAO inhibitor, PLZ, attenuated apomorphine-induced locomotor suppression in the absence of any changes in [³H]GBR 12935 binding to DA uptake sites. This suggested that the effects of chronic antidepressant treatment may be mediated through the DA system, not by decreasing the number of DA uptake sites as previously proposed by Nomikos *et al.* (1991), but by decreasing presynaptic DA autoreceptor function.

In chapter 4, DMI and PLZ, in addition to the irreversible, selective type-A MAO inhibitor, CLG, attenuated the locomotor-suppressant effects of quinpirole, suggesting that these antidepressants may mediate their effects by decreasing DA autoreceptor function. The results of these experiments, therefore, suggest that these antidepressants may mediate their effects by decreasing presynaptic DA autoreceptor function.

The attenuation of yawning induced by quinpirole, a D₃/D₂ DA receptor agonist, following chronic PLZ and CLG treatment and the absence of this effect following chronic DMI or CMI treatment suggests that the TCAs may mediate their effects on locomotor suppression by the involvement of a different DA receptor

subtype than do the MAO inhibitors. The discrepancy between the effect of DMI on quinpirole-induced locomotor suppression and yawning suggests that DMI may even mediate its effects on these two behavioural responses through different receptor subtypes. The difference between the effects of TCAs (DMI) and the MAO inhibitors (PLZ and CLG) on apomorphine- and quinpirole-mediated effects may be related to their different downregulation of D₁-like (DMI) *versus* D₂-like (PLZ and CLG) receptors (Paetsch and Greenshaw, 1992). None of these antidepressants had any effect on the grooming induced by SKF 38393, a D₁-like DA receptor agonist.

Despite their selectivity for the 5-HT system, several lines of evidence suggest that SSRIs may mediate some of their effects by the DA system. CMI, which is a potent but not selective 5-HT uptake inhibitor, is used to treat obsessive-compulsive disorder (Hyman *et al.*, 1995), the symptoms of which are to some extent similar to the stereotypy induced by the stimulation of the DA system (Zohar and Insel, 1987). In the experiments presented in chapters 3 and 4, respectively, CMI did not attenuate the locomotor-suppressant effects of apomorphine and quinpirole, respectively, suggesting that antidepressants which are potent inhibitors of 5-HT uptake may not mediate their effects by decreasing presynaptic DA autoreceptor function. Since CMI is a potent but not selective 5-HT uptake inhibitor, the effects of FLU, a SSRI, on the locomotor-suppressant effects of quinpirole were assessed in chapter 6. The lack of attenuation of quinpirole-induced locomotor suppression following chronic administration of FLU strongly suggested that SSRIs do not mediate their effects by decreasing presynaptic DA autoreceptor function.

A recent study by Simon and Appel (1997), which reports the ability of rats to discriminate cocaine from saline, suggests that FLU may mediate some of its effects by the DA system. The results of the study presented in this thesis are consistent with those of Collu *et al.* (1997) who also reported a lack of attenuation of FLU on quinpirole-induced locomotor suppression. Thus, these results suggest that if FLU does mediate some of its effects through the DA system, it does not appear to do so by decreasing presynaptic DA autoreceptor function.

The affinity of TMP for D₂ DA receptors (Gross *et al.*, 1991) and its ability, following acute treatment, to increase DA synthesis and DOPA accumulation (Carlsson and Lindqvist, 1978) suggests that TMP, an antidepressant whose mechanism of action is currently unknown, may mediate its effects through the DA system. TMP did not attenuate the locomotor-suppressant effect of quinpirole, suggesting that it does not mediate its effects by decreasing presynaptic DA autoreceptor function.

The attenuation of quinpirole-induced locomotor suppression following chronic s.c. administration of CLG in chapter 4 suggested that selective inhibition of MAO-A may be a sufficient condition for mediating the functional downregulation of presynaptic DA autoreceptors. It was demonstrated in chapter 5, however, that chronic oral administration of the reversible MAO inhibitors, MOC and BROF, as well as the irreversible MAO inhibitors, PLZ and CLG, did not attenuate the apomorphine-induced locomotor suppression. These results with MOC and BROF were consistent with the reported lack of change in affinity or density of either D₁ or D₂ DA receptors following chronic oral administration of these MAO inhibitors

(Klimek *et al.*, 1990). The results with PLZ and CLG, however, were inconsistent with previous studies (Allison *et al.*, 1993;1995) which demonstrated that chronic s.c. administration of PLZ and CLG attenuated the locomotor-suppressant effects of apomorphine (PLZ) and quinpirole (PLZ and CLG), despite levels of MAO inhibition which were comparable to those following chronic oral administration. These results suggested that MAO inhibition may not be a sufficient condition for mediating PLZ's effects. Other studies (McManus *et al.*, 1991; Paetsch *et al.*, 1993) have previously reported that following different doses of chronically administered (s.c.) PLZ, the levels of MAO inhibition were comparable but the levels of brain and liver PEA were significantly different. These results suggested a possible role of PEA in mediating the effects of PLZ. Therefore, the effects of higher (17 and 30 mg kg⁻¹) doses of PLZ on apomorphine-induced locomotor suppression were assessed and the brain and liver concentrations of PEA were measured in order to determine if PEA plays a role in mediating the effects of PLZ on DA autoreceptor-mediated functional responses. Although there was a significant difference between the brain PEA levels obtained following the 17 and 30 mg kg⁻¹ doses of PLZ, the extent to which these two doses of PLZ attenuated apomorphine-induced locomotor suppression did not differ. This lack of a dose-response relationship of PLZ suggests that an increase in PEA levels is not a sufficient condition for mediating the effects of PLZ. Increased GABA and biogenic amine levels also do not appear to be a sufficient condition for mediating the effects of PLZ since they had no relationship to the extent to which these two doses attenuated the locomotor-

suppressant effect of apomorphine.

It has been suggested that a metabolite, derived primarily from the metabolism of PLZ by MAO-B, may be important in the elevation of GABA levels (Todd and Baker, 1995). Since the 30 mg kg⁻¹ dose of PLZ resulted in significantly higher levels of GABA than did the 17 mg kg⁻¹ dose, it may be that the levels of the metabolite responsible for increasing GABA levels are increased following hepatic first-pass metabolism. These two doses of PLZ attenuated apomorphine-induced locomotor suppression to the same extent; therefore, it seems that increased levels of GABA and the metabolite which enhances GABA levels are also not a sufficient condition for mediating PLZ's effects on DA autoreceptor function.

Presynaptic autoreceptors are involved in regulating the release of neurotransmitters such as DA. The release experiment is a procedure which allows us to measure the ability of DA receptor agonists to alter DA release and, consequently, to identify their selectivity for presynaptic DA autoreceptors (Baker and Dyck, 1985). The superfusion technique permits the study of neurotransmitter release from nerve terminals without the interference of reuptake of the neurotransmitter from the synaptic cleft once released (Raiteri *et al.*, 1974). The superfusion study in chapter 6 was intended to be a preliminary part of an experiment which would more clearly assess the affinity of apomorphine and quinpirole for D₂ versus D₃ receptors, and subsequently, determine the DA receptor subtype through which the antidepressants discussed in this thesis may be mediating their effects. Unfortunately, my results did not support the use of K⁺ stimulation as a reliable model with which to assess the presynaptic DA

autoreceptors in rat nucleus accumbens prisms.

Although the 3 μ M concentration of apomorphine decreased 15 mM K⁺-stimulated [³H]DA release in nucleus accumbens prisms, there was no evidence of this inhibitory effect by apomorphine at any of the other concentrations tested. It may be that K⁺-stimulation is not as comparable physiologically as electrical stimulation as a technique with which to assess the presynaptic DA autoreceptors. There is also the possibility that brain prisms may not offer an environment which resembles the normal physiological environment as much as that which is obtained with brain slices.

Overall, the results of the experiments presented in this thesis suggest that some antidepressants (DMI, PLZ and CLG) may mediate their effects via the DA system by decreasing presynaptic DA autoreceptor function. For other antidepressants, such as CMI and FLU, it appears that if they do mediate their effects through the DA system, it is not by decreasing presynaptic DA autoreceptor function. Although CMI and FLU, potent 5-HT uptake inhibitors, did not alter the functional response to DA receptor agonists in these experiments, the DA system as a mediator of their effects should not be completely ruled out.

In summary, the experiments outlined in this thesis have addressed some important issues and have advanced our knowledge with respect to the role of DA in mediating the effects of different antidepressants. The evidence which supports the role of DA in mediating the rewarding properties of stimuli and the presence of anhedonia as a core symptom of depression, suggests that the DA system may play a very important role in the etiology and/or treatment of depression. Furthermore,

the ability of chronic antidepressant treatment to potentiate the stimulatory and attenuate the suppressant functional responses to DA agonists suggests that antidepressants may be mediating their effects through the DA system.

It is possible, however, that some antidepressants do not mediate their effects by enhancing DA neurotransmission directly and that those which do may not mediate their effects exclusively through the DA system. Furthermore, in view of the different biochemical effects of different antidepressants, and the different subtypes of depression, it is important to consider that there may be different neurotransmitter systems involved in both the etiology and treatment of these different subtypes. Although it is important not to assume a single-model approach to understand the basis for the psychopathology of depression, it appears that the DA system may play an important role in the etiology and treatment of depression and shows promise as a target for further research regarding antidepressant drug action.

9.2. REFERENCES

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